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Functional brain imaging of facial emotion processing in individuals with intellectual impairments.

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Table of Contents

Abstract

From the relatively early descriptions of fragile X syndrome, it was recognised that there were differences in social communication, which overlapped or mirrored those seen in idiopathic autism. And in parallel, genetic screening of individuals with autism revealed fragile X syndrome as a leading inherited cause of autism. Reviewing the literature of both of these conditions; it is obvious that although as clinical entities they include individuals with varying degrees of co-occurring intellectual disabilities or impairments, the existing literature has largely included those who are more, or most, intellectually able. This is particularly so in brain imaging research, largely by reason of the significant challenges of the imaging environment, with many of the challenges of having a scan corresponding with particular difficulties for the individuals; e.g. the noise of the scanner being of particular difficulty for those with sensory hypersensitivities (common in fragile X syndrome and autism).

In the current study, functional brain imaging was used to investigate the role of autism in the processing of facial emotions in two cohorts – one with special educational needs and one with fragile X syndrome. Particular consideration was given to whether the emerging patterns of activations in any way mirrored those seen in the extant literature and whether to any degree it could be considered that autism in the context of lower cognitive ability (be that by virtue of idiopathic intellectual impairment or a known single-gene disorder) has the same underlying neural correlates as in individuals of average or above average intellect.

In a group of individuals with special educational needs, it was found that those with high autistic traits had a region of hyperactivation to neutral faces in the right rolandic operculum; replicating a finding previously described in a meta-analysis of prior functional imaging studies in individuals with autism and of average or enhanced cognitive ability. In parallel, the sub-group with low

autistic traits had a cluster of significantly greater activation in the left supramarginal gyrus / angular gyrus in response to fearful facial stimuli compared to the autistic sub-group. This pattern of relative hypo-activation in individuals with autism to emotional stimuli is typical of the existing literature in autism and adds further weight to the idea that, at least in part, individuals with autism of lower cognitive ability show similar changes in neural function.

In a group of individuals with fragile X syndrome, those with high autistic traits had a cluster of significantly lower brain activation in the left superior temporal gyrus / left supramarginal gyrus in response to fearful faces when compared to those with low autistic traits. This cluster overlapped previous findings in both the fragile X literature, but also prior work in the broader autism literature, suggesting that autism in the context of a monogenic form of ID may have similar neurobiological correlates as seen in idiopathic autism.

The results from this study show firstly that imaging individuals with significant cognitive impairments is feasible. Secondly, the results suggest that autistic individuals who have concurrent intellectual impairments share some of the same patterns of brain function as seen in autistic individuals of average or enhanced cognitive ability, who are most commonly recruited for brain imaging studies. Finally, the results suggest that autistic traits in the context of fragile X syndrome are associated with brain activation differences, which overlap those previously described in idiopathic autism. Further research is necessary to quantify the nature and degree of this overlap more fully.

Lay summary

Fragile X syndrome is the most common cause of inherited intellectual disability and autism. From the relatively early descriptions of fragile X syndrome, it was recognised that there were differences in social communication, which overlapped or mirrored those seen in autism, where the cause is unknown. And in parallel, genetic testing of groups of individuals with autism revealed that fragile X syndrome was a leading cause of autism. Much of the existing research on these conditions has largely included those who are more, or most, intellectually able. This is particularly so in brain imaging research, largely by reason of the significant challenges of the imaging environment, with many of the challenges of having a scan corresponding with particular difficulties for the individuals; e.g. the noise of the scanner being of particular difficulty for those who find loud noises very difficult to tolerate (which is common in fragile X syndrome and autism).

In the current study, brain imaging was used to investigate the role of autism in two cohorts – one with special educational needs and one with fragile X syndrome. During the brain imaging, it was of interest as to whether the autistic group had different patterns of brain activity to the non-autistic group. Particular consideration was given to whether the emerging patterns of brain activity in any way mirrored those seen in the existing literature and whether to any degree it could be considered that autism in the context of lower intellectual ability has the same underlying patterns of brain activity as in individuals of average or above average intellect.

In a group of individuals with special educational needs, it was found that those with high autistic traits had a region of increased brain activity when looking at pictures of neutral faces in a particular part of the brain on the right hand side; replicating a finding previously described in a study from 2012 that had summarised all the prior research in individuals with autism (mainly of average

or enhanced intellectual ability). In parallel, the sub-group with low autistic traits had a cluster of significantly greater activation on the left hand side of the brain in response to viewing pictures of fearful faces in the scanner compared to the autistic sub-group. This pattern of relative lower brain activity in individuals with autism to highly emotional stimuli is typical of the existing literature in autism and adds further weight to the idea that, at least in part, individuals with autism of lower cognitive ability show similar changes in brain function.

In a group of individuals with fragile X syndrome, those with high autistic traits had a cluster of significantly lower brain activation on the left hand side of the brain in response to fearful faces when compared to those with low autistic traits. This region of lower activity overlapped previous findings in both the fragile X literature, but also prior work in the broader autism literature, suggesting that autism in the context of a genetic form of intellectual disability may have similar patterns of brain activity as seen in individuals with autism of unknown cause.

The results from this study show firstly that imaging individuals with significant cognitive impairments is feasible. Secondly, the results suggest that autistic individuals who also have intellectual impairments share some of the same patterns of brain function as seen in autistic individuals of average or enhanced cognitive ability, who are most commonly recruited for brain imaging studies. Finally, the results suggest that autistic traits in the context of fragile X syndrome are associated with brain activation differences, which overlap those previously described in idiopathic autism. Further research is necessary to quantify the nature and degree of this overlap more fully.

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Declaration

I declare that this thesis has been composed by me, and that the work is my own, except where clearly indicated.

I confirm that the work has not been submitted for any other degree or professional qualification.

The publications included are my own work, and my contributions are clearly annotated in the publications where appropriate.

Andrew G. McKechanie

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Glossary of abbreviations

ABIDE	Autism Brain Imaging Data Exchange
ADHD	Attention Deficit Hyperactivity Disorder
ADOS	Autism Diagnostic Observation Schedule - K; Korean Translation
ADI-R	Autism Diagnostic Interview – Revised - K; Korean Translation
AGG	Adenine Guanine Guanine
ALE	Activation Likelihood Estimation
AS	Asperger Syndrome
ASD	Autism Spectrum Disorder
BA	Brodmann Area
BOLD	Blood Oxygen-Level Dependent
CARS	Childhood Autism Rating Scale
CDC	Centers for Disease Control and Prevention
CGG	Cytosine Guanine Guanine
CNV	Copy Number Variation
CRIC	Clinical Research Imaging Centre
CSS	Calibrated Severity Score
dACC	Dorsal Anterior Cingulate Cortex
dB(A)	A-weighted decibels
DD	Developmental Delay
DICOM	Digital Imaging and Communications in Medicine
DISCO	Diagnostic Interview for Social and Communication Disorders
DSM	Diagnostic and Statistical Manual of the American Psychiatric Association - III; Third edition - IV; Fourth Edition - IV-TR; Fourth Edition, Text Revision - 5; Fifth Edition
EEG	Electroencephalogram
EMG	Electromyography
EPI	Echo-Planar Imaging

ERP	Evoked Response Potential
fALFF	Fractional Amplitude of Low-Frequency Fluctuations
FFA	Fusiform Face Area
FG	Fusiform Gyrus
fMRI	Functional Magnetic Resonance Imaging
FMR1	Fragile X Mental Retardation 1 gene
FMRP	Fragile X Mental Retardation Protein
FOV	Field-Of-View
FSIQ	Full-Scale IQ
FWHM	Full Width Half Maximum
FXS	Fragile X syndrome
FXTAS	Fragile X-associated Tremor-Ataxia Syndrome
FXPOI	Fragile X-associated Premature Ovarian Insufficiency
FXSoc	Fragile X Society
GSR	Global Signal Regression
HFA	High-Functioning Autism
HVCP	High Verbal and Cognitive Performance
IASSMD	International Association for the Scientific Study of Mental Deficiency
ICD	International Classification of Disease - 10; Tenth Edition - 11; Eleventh Edition
ID	Intellectual Disability
IFG	Inferior Frontal Gyrus
IQ	Intelligence Quotient
K-BIT	Kaufmann Brief Intelligence Test
k_E	Extent of cluster in voxels
LVCP	Low Verbal and Cognitive Performance
MD	Mental Deficiency
MEG	Magnetoencephelogram
MNI	Montreal Neurological Institute
MOMX	Macro-Orchidism-Marker X

mPFC	Medial Prefrontal Cortex
MPRAGE	Magnetization Prepared - RApid Gradient Echo
MR	Mental Retardation
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NHS	National Health Service
NMR	Nuclear Magnetic Resonance
PCR	Polymerase Chain Reaction
PDD-NOS	Pervasive Developmental Disorder, Not Otherwise Specified
PDM	Public Domain Mark
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
$p_{FWE\text{-}corr}$	p-value of significance, corrected for family-wise error
PIQ	Performance IQ
PQBP1	Polyglutamine Binding Protein 1 gene
rCBF	Regional cerebral blood flow
rCMR	Regional cerebral metabolic rate
REC	Research Ethics Committee
RF	Radio-Frequency
RMSD	Root-Mean square Deviation
RNA	Ribonucleic Acid
ROI	Region of Interest
RRB	Restricted and Repetitive patterns of Behaviour and interest
rs-fMRI	Resting State Functional Magnetic Resonance Imaging
SCQ	Social Communication Questionnaire
SEN	Special Educational Needs
SFARI	Simons Foundation Autism Research Initiative
SMA	Supplementary Motor Area
SPL	Sound Pressure Level

SPM	Statistical Parametric Mapping
SRS	Social Responsiveness Survey
STG	Superior Temporal Gyrus
STS	Superior Temporal Sulcus
SVC	Small Volume Correction
TD	Typically-Developing
TE	Echo Time
ToM	Theory of Mind
TR	Repeat Time
UTR	Untranslated Region
VABS	Vineland Adaptive Behaviour Scale
VIQ	Visual IQ
VLPFC	Ventrolateral Prefrontal Cortex
WAIS	Wechsler Adult Intelligence Scale
WISC	Wechsler Intelligence Scale for Children
($Z_=$)	Z-score

List of tables

List of figures

List of plates

Overview of thesis

This thesis is primarily concerned with examining whether existing findings of imaging studies in autism and fragile X syndrome can be generalised to individuals with greater degrees of intellectual impairment and what adaptations may be necessary to facilitate their participation in imaging research. Chapter 1 is an introduction to fragile X syndrome and autism. Chapters 2 and 3 are systematic reviews of functional MRI studies in autism and fragile X syndrome respectively and discuss the problem of selection bias towards those who are of average or enhanced cognitive ability. Chapter 4 covers the materials and methods used in the two functional imaging experimental chapters. Chapter 5 is a functional imaging investigation of implicit emotion processing in a group of individuals of lower cognitive ability, with an interest in how autistic traits affect such processing. Chapter 6 is a functional imaging investigation of implicit emotion processing in a group of individuals with fragile X syndrome, with an interest in how autistic traits affect such processing. Chapter 7 is a general discussion of the findings, along with discussion of the limitations of the studies and suggestions for future research.

There is a focus throughout on the challenges facing individuals who are less cognitively able for their inclusion in research and the generalizability of findings in the broader research to them.

Chapter 1: An introduction to fragile X syndrome, autism and their intersection.

Introduction

History should have taught us the lessons of how narrow inclusion criteria in research can put at risk the later generalizability of the findings[1]. However, the issue persists in the field of neurodevelopmental disorder research. There remains a certain disconnect in current investigation in this field, with many studies examining only those who are the most cognitively able; whilst in parallel, narrowly-defined groups of individuals, often with specific genetic conditions and a greater degree of intellectual impairment, are recruited for controlled trials of new medications in the hope that one day the drugs may be available to a much wider market. Such a disconnect is problematic and without any evidence that findings in one group hold across a spectrum of cognitive ability there is a risk that our understanding of the nature of such developmental disorders will be limited.

The particular fields of interest to the author are autism, fragile X syndrome and their intersection; and so before considering imaging studies of these in more depth it is necessary to first set the scene by considering the current understanding of these as diagnostic entities. In Waddington's epigenetic

[1] The well-intended guidance from the FDA in 1977 that, "women of childbearing potential should be excluded from the earliest dose ranging studies" (United States Department of Health; Education and Welfare; Food and Drug Administration; Bureau of Drugs, 1977) which followed the thalidomide and diethylstilbestrol health concerns almost certainly contributed to narrow recruitment in clinical trials (A. L. Herbst, Ulfelder, & Poskanzer, 1971; Kuenssberg, Simpson, & Stanton, 1961). The consequence of this came to the fore with the previously undetected difference in metabolism of zolpidem between men and women; which was later linked to morning-after drowsiness and driving accidents and led to revised dosing guidance (United States Food and Drug Administration, 2013). It has only been since 2016, that the National Institutes of Health (NIH) have expected that sex is taken into account as a biological variable in clinical studies (National Institutes of Health, 2015).

landscape (Waddington, 1957) one genetic starting place can lead to multiple possible endpoints, and this is of direct relevance to fragile X syndrome. Whereas in autism it is almost the mirror-image that is seen: a phenotype that serves as the diagnostic criteria, yet subserved by multiple possible aetiologies, including fragile X syndrome.

Introduction to fragile X syndrome

Fragile X syndrome is the most common inherited form of intellectual disability and an important cause of autism. It is caused by the dysfunction or absence of the Fragile X Mental Retardation Protein (FMRP), a protein important in the regulation of a large number of post-synaptic RNAs. The absence of FMRP is most commonly caused by an expansion of a CGG repeat sequence in the 5' untranslated region (UTR) of the FMR1 gene on the X chromosome, in turn leading to transcriptional silencing. In boys this leads to a characteristic syndrome of intellectual disability combined with a number of common physical features. Whereas, in girls the presentation is more variable due to random inactivation of the X chromosome; thus in girls intellectual functioning varies from the typical range to those with significant intellectual disability.

As will be explored later, early accounts of fragile X syndrome remarked on the frequent presence of autistic traits. In more recent times, these ideas have evolved with parallel ideas emerging as to the nature of this overlap of presentation; raising the interesting question of whether the autism seen in FXS is the same as idiopathic autism.

History of fragile X syndrome

Early history of X-linked mental retardation

Prior to the discovery of fragile X syndrome, it had long been recognised that intellectual disability was more common in males. William Ireland, former superintendent at the Royal Scottish National Hospital in Scotland, had reported carefully on the reported prevalence of intellectual disability (then, idiocy) from accounts of colleagues across Europe and The United States of America (Ireland, 1877). In the majority of cases an excess of males was reported, although in a later text he supposed that this may relate to increased cranial diameter in boys and the likelihood of a traumatic delivery:

> "It seems likely that the larger size of the head of the male infant, which renders it more liable to compression and injury at parturition, as shown by Sir James Simpson, is the cause of the higher mortality of male children during the first year of life, and especially of their greater liability to diseases of the brain." (Ireland, 1898)

Johnson had described in 1895 an excess of some 24% males in a sample of children with mental retardation (Johnson, 1895), with similar, later reports from Luxenberger (1932) and Penrose (1938). Albeit that, in a later discussion of the results of his earlier study, Penrose (1963) reflected that he felt the excess could be explained by ascertainment bias linked to excess aggression in the males and was not entirely convinced that there was a genuine sex-linked excess.

In 1922 Ash described 11 cases of congenital blindness from microphthalmia in one family (Ash, 1922). Other than one individual noted to have epilepsy, there were no other comorbidities reported at that time. What was noted, however, was the pattern of inheritance; affectedness being confined to males, but apparently transmitted through healthy females. In 1937, Roberts reported on an extended pedigree of this original family, by which time more children had been born and more in-depth investigation possible (Roberts, 1937). By now it was evident that the sex-linked condition originally described was also associated with 'mental deficiency'. Thus, the family reported by Ash in 1922 likely represents one of the earliest clear descriptions of sex-linked intellectual disability; even if retrospective analyses of earlier kindreds have shown a pattern of inheritance consistent with x-linked inheritance (e.g. those described by Dugdale (1877) and Goddard (1912)).

On this backdrop, it was in 1943 that Martin and Bell published their article on "A pedigree of mental defect showing sex-linkage" (J. P. Martin & Bell, 1943), which is now recognised as the first clear description of what we know as fragile X syndrome. In their original paper they reported on 11 individuals described to have imbecility, by virtue of a severe dementia, without any common physical abnormalities. In addition to the 11 males described, they also reported on 2 females in the same family, one of whom was described as having milder degrees of mental deficiency. All affected individuals were noted to have very significant impairment of expressive language, reported as, "the

very imperfect development of the speech function". In addition to the intellectual and language impairment noted, two of the individuals were reported as having "pronounced psychotic traits"; one of a paranoid type, the other of a schizoid type. This early report was followed over the next two and a half decades by a variety of further reports of sex-linked mental retardation (Allan, Herndon, & Dudley, 1944; Dunn et al., 1963; Losowsky, 1961; Renpenning, Gerrard, Zaleski, & Tabata, 1962).

Identification of the genetic origin of fragile X syndrome

It was then in 1967 that Lubs discovered an unusual appearance to the X chromosome in some of the metaphase figures of a subject, concluding that,

"either the secondary constriction itself or a closely linked recessive gene may account for the pattern of X-linked inheritance" (H. A. Lubs, 1969);

However, it wasn't until 1984 that it was confirmed that the phenotype of this individual matched that as described by Martin & Bell (H. Lubs, Travers, Lujan, & Carroll, 1984).

Although Lubs had made this observation in 1967, it was in 1970 that a paper on X-linked mental retardation by Escalante was presented at the second congress of the International Association for the Scientific Study of Mental Deficiency (IASSMD) by Dr J.M. Opitz at the request of the authors, due to their absence (Escalante, Grunspun, & Frota-Pessoa, 1970). In it, Escalante described a family with nine affected males with severe mental retardation and enlarged testes and scrotum in seven of the nine. The inheritance was also noted to be 'typically X-linked" and that, "this seems to be a new syndrome". Separately, in Escalante's PhD thesis (Escalante, 1971) and a later book chapter (Escalante & Frota-Pessoa, 1973); another family with three brothers with moderate-severe ID and two sisters with mild ID were described, notably associated with the presence of a marker C chromosome with a subterminal constriction. Twice in the 1973 book chapter, Escalante speculates that the marker C chromosome is actually the X chromosome:

"Como as duas mulheres que apresentam a constrição têm DM de grau leveou limítrofe, é provável que o cromosomo a anômalo seja um X" (Since the two women with the constriction have mild or borderline MD [mental deficiency], the anomalous chromosome is likely to be an X)

"Vê-se um cromosomo C…, provavelmente um X, com constrição subterminal, cuja presença está associada a DM" (You see a C chromosome, probably an X, with sub-terminal constriction, whose presence is associated with MD (mental deficiency)) .

In 1978 Turner reported having identified that all males with the fragile site on the X chromosome also had macro-orchidism (Turner, Till, & Daniel, 1978). This was followed by a string of similar publications reporting on further pedigrees with the marker X chromosome, intellectual disability and macro-orchidism (Howard-Peebles, 1980; Howard-Peebles & Stoddard, 1979; Jacobs et al., 1979; Sutherland & Ashforth, 1979; Turner, Daniel, & Frost, 1980). Despite the frequent co-occurrence of macro-orchidism, it was noted that it was not ubiquitous (Howard-Peebles & Stoddard, 1980). In 1980 Turner & Opitz suggested that the eponym of Martin and Bell (which at this stage was associated with sex-linked MR, but not as yet with the fragile site identified by Lubs) should be discarded as "the exact diagnosis in the family reported by Martin and Bell [1943] is not known". Instead, Turner & Opitz suggested for the name "the macro-orchidism-marker X (MOMX) syndrome" to be used (Turner & Opitz, 1980).

In parallel, other previously described kindreds were subsequently identified as being associated with fragile X site (D. S. Herbst, 1980; Jacobs et al., 1980),

whereas, the family described by Renpenning (who had been described as having a different physical phenotype) were confirmed not to have the fragile site at Xq27.3 (Fox, Fox, & Gerrard, 1980). Rather, it was shown to map to Xp11.2 (Stevenson et al., 1998) and ultimately due to a mutation in the PQBP1 gene on the X chromosome at Xp11.23 (Lenski et al., 2004).

In 1981, having re-contacted a number of the original family members described, Richards confirmed that the family originally described by Martin and Bell (1943) did, in fact, have the fragile site visible on microscopy; and proposed that the condition should be designated, “The Martin-Bell Syndrome” (Richards, Sylvester, & Brooker, 1981). A year later, Vianna-Morgante suggested that by virtue of Escalante having been first to describe X-linked macro-orchidism associated with MR and second to describe the association of the fragile site with X-linked MR in males in females, that he should instead be afforded the eponym (Vianna-Morgante, Armando, & Frota-Pessoa, 1982). Despite the back-and-forth of discussing the best nomenclature for the syndrome, it was ultimately fragile X syndrome which emerged as the dominant descriptor.

Characterisation of the fragile site

Following the discovery of a fragile site by Lubs in 1967, the precise location was further refined over the ensuing decade (Giraud, Ayme, Mattei, & Mattei, 1976; Harvey, Judge, & Wiener, 1977; Sutherland, 1977); with Harrison's electron microscopy study better visualising the so-called fragile site (Harrison, Jack, Allen, & Harris, 1983). However, it wasn't until 1991 that the gene responsible for FXS was identified (Bell et al., 1991; Oberle et al., 1991; Verkerk et al., 1991; Yu et al., 1991). This having been identified, diagnosis is now most reliably made with a combination of PCR and Southern blotting techniques.

From the early descriptions of fragile X syndrome, the syndrome is now much more fully described, with a range of physical, neurological, cognitive, behavioural and developmental features being clear, which will be described further on.

Epidemiology

Early studies of prevalence (J. Murray, Cuckle, Taylor, & Hewison, 1997; Pembrey, Barnicoat, Carmichael, Bobrow, & Turner, 2001) suggested that prevalence estimates of the full mutation in the general population were in the region of 1:4000 in males and 1:8000 for females. However, a recent systematic review and meta-analysis by Hunter et al (2014) reported population frequencies of the full mutation at 1.4/10,000 for males and 0.9/10,000 for females. Their estimates were based on total population and non-ID population screening by PCR & Southern blotting, and sought to address some of the concerns about previous estimates, namely reducing the problems associated with extrapolating from small, selected samples. For the premutation, Hunter et al reported estimates of 1:300 for females and 1:850 for males.

Given what is known of the genetics of fragile X; the population prevalence of the premutation and the full mutation do not add up. It is likely that there will be multiple explanations for this, however, it is known that premutation carriers identified through screening programmes are less likely to go on to have a child with a full mutation than a mother identified as having a premutation by virtue of having a child with FXS. Possible explanations for this disparity include AGG interruptions to the CGG sequence, other genetic background factors or early loss of FXS pregnancies.

Features of fragile X syndrome

Cognitive features

The feature that was first identified by Martin and Bell (J. P. Martin & Bell, 1943) was that of significant intellectual impairment. Although the measures for this were somewhere between non-existent and crude at that time, their description of imbecility was subsequently folded into DSM and ICD descriptions of moderate or severe mental retardation or intellectual disability (i.e. those with an IQ of approximately 20-49). More recent and detailed examination of the distribution of IQs of those with fragile X syndrome shows that in 65-85% of male children the average IQ is <55 (Hessl et al., 2009), with this gradually decreasing over time to a mean of about 40 by the time of adulthood (Dykens, Hodapp, & Leckman, 1989). While in general, males tend to be more severely affected, a degree of variability is still seen; a combination of mosaicism, methylation mosaicism, polygenic background and environmental factors all playing their part. For females with a full expansion of the FMR1 CGG repeat, the average IQ is about 80. It should be noted, however, that whilst the average IQs are quite distinct, the full range of IQs can be seen in females, due to random X-inactivation. As Howe (1848) had reflected, when discussing "transmission of hereditary tendencies to disease of mind and body";

"it may affect one child more, and another less; it may affect one in one form and another in another…"

Quintin et al (2016) examined the cognitive profiles of male and female children aged 6-16 with FXS and replicated the finding of a widening gap between those with FXS and their normative sample in the areas of verbal comprehension, perceptual organisation and processing speed; 3 of the 4 indices of the WISC-III assessment. Interestingly they showed that on freedom from distractibility, the fourth index, that between ages 6 and 16 the gap between those with FXS and their normative sample narrowed.

Language development

In addition to the cognitive impairments commonly seen in individuals with FXS, much has been done to explore whether language is differentially affected. The evidence does not all agree, however, it appears that receptive language development broadly parallels that of the individual's cognitive development (Abbeduto, Brady, & Kover, 2007). However, expressive language is more commonly impaired with both delays in its development and slower rates of growth compared to younger children matched for nonverbal cognitive level (Estigarribia, Martin, & Roberts, 2012; G. E. Martin et al., 2012). Beyond simple measures of language development, further analysis reveals more subtle changes with shorter utterances, lower grammatical complexity and fewer noun and verb phrases (Thurman, McDuffie, Hagerman, Josol, & Abbeduto, 2017).

Physical features

Although in the original description by Martin and Bell, they noted the absence of any physical features associated with the intellectual disability, it has subsequently become clear with collation of phenotypic information across individuals and cultures that there are physical features associated with the syndrome. Macro-orchidism was one of the earliest common features to be described (Escalante et al., 1970); a feature that may be present at birth (Cantu et al., 1976). Further common features include a high forehead, increased head circumference in childhood, enlarged ears, a narrowly arched high palate, flat feet and connective tissue abnormalities, including hyper-extensible joints. Interestingly, although it is not possible to confirm genetically, there are early images of individuals with intellectual disability, high palates and large foreheads who bear significant resemblance to individuals with FXS (McKechanie et al, Appendix 1).

Medical features

Alongside the more physical features described, there are a number of common medical features (Kidd et al., 2014). These are summarised below.

Neurological features

Epilepsy is seen in up to 20% of males with FXS, and a lower proportion of affected females. This generally presents as a focal epilepsy, which is noted to usually be relatively responsive to medication (Berry-Kravis, 2002).

Cardiac problems

Mitral valve regurgitation and prolapse have been noted to be more prevalent in FXS, although estimates for mitral valve prolapse vary very widely from 0.5% (Kidd et al., 2014) to 77% (Puzzo et al., 1990). Less common, related abnormalities also include aortic and pulmonary root dilatation and tricuspid septal leaf prolapse.

Gastrointestinal problems

Gastro-oespophageal reflux is reported in approximately 11% of those with FXS (Goldson & Hagerman, 1993; Utari et al., 2010), although it should be noted that reflux is also more common in individuals with intellectual disability more generally (Hermans & Evenhuis, 2014), with incidence increasing with severity of ID; occurring in up to 50% of those with severe or profound ID (van Timmeren, van der Putten, van Schrojenstein Lantman-de Valk, van der Schans, & Waninge, 2016).

Ear, nose & throat problems

Otorhinolaryngeal problems are frequently reported, with recurrent otitis media reported in 53% of individuals with FXS (Kidd et al., 2014) and recurrent sinusitis reported in up to 23% of individuals (Randi Jenssen Hagerman & Hagerman, 2002) being the most common complaints.

Visual problems

A variety of ocular and visual problems, including refractive errors, nystagmus and strabismus, are reported as being more common in FXS. One of the problems that arise, however, in trying to establish good data on this is that thorough eye examination is made difficult by the anxiety, hyperactivity and attention problems commonly seen in FXS (Hatton, Buckley, Lachiewicz, & Roberts, 1998).

Musculoskeletal problems

A variety of musculoskeletal abnormalities are noted in FXS, with pes planus, excessive joint laxity and scoliosis being commonly noted (Davids, Hagerman, & Eilert, 1990). These findings follow on from the early finding of Opitz of pronounced hyperextensibility in an individual with FXS (Opitz, Westphal, & Daniel, 1984). Hypotonia, especially noted in childhood but improving with age is also a common finding (Lachiewicz, Dawson, & Spiridigliozzi, 2000). This may

compound difficulties with co-ordination and in some individuals is associated with feeding and swallowing difficulties.

Developmental features

ADHD

From early descriptions of children with intellectual disability, some were noted to be, "Endowed with … considerable energy in many cases…" (Johnson, 1895). More specifically, hyperkinetic behaviour and poor attention are described as common features in FXS. Some early case series reported these features in all members of their series (Bregman, Leckman, & Ort, 1988); whilst Fryns, Jacobs, Kleczkowska, & van den Berghe (1984) note that,

"The most striking behavioural problem is hyperactivity together with concentration difficulties".

Sullivan et al (2006) reported 59% of children with FXS as having ADHD, based on parent and teacher reports, while in their survey of parents Bailey et al (2008) reported that 84% of males and 67% of females with FXS had been diagnosed or treated for attention problems. However, Quintin (2016) reports that distractibility generally improves throughout childhood.

Autism

It was recognised in some of the early descriptions of series of confirmed fragile X syndrome males that there were higher levels of social, communication and sensory difficulties than could be accounted for by level of intellectual disability alone (Borghgraef, Fryns, Dielkens, Pyck, & Van den Berghe, 1987; Brown et al., 1982; Cohen et al., 1988; Fryns, 1984; R. J. Hagerman, Jackson, Levitas, Rimland, & Braden, 1986; Hanson, Jackson, & Hagerman, 1986; Levitas et al.,

1983; Meryash, Szymanski, & Gerald, 1982; Wolf-Schein et al., 1987). In parallel, early studies in which groups with autism were screened for fragile X reported 0-16% of autistic males with fragile X syndrome (Brown et al., 1986; Goldfine et al., 1985; Mcgillivray, Herbst, Dill, Sandercock, & Tischler, 1986; Pueschel, Herman, & Groden, 1985; Venter, Op't Hof, Coetzee, Van der Walt, & Retief, 1984; Wahlstrom, Gillberg, Gustavson, & Holmgren, 1986; M. S. Watson et al., 1984). Smalley et al (1988) in their pooled analysis of previous studies reported 8% of males with autism (48/613) as having fragile X as their genetic diagnosis. With autism now being more widely recognised especially in those without an intellectual disability, these estimates are consequently lower, with fragile X syndrome accounting for perhaps only 0.5% of idiopathic autism (McGrew, Peters, Crittendon, & Veenstra-VanderWeele, 2012; Roesser, 2011; Shen et al., 2010).

Despite these associations between features of autism, autism diagnosis, and fragile X syndrome, the association is still not wholly clear and in many cases the presentation of ASD in the context of FXS differs from the presentation in idiopathic ASD. This will be explored in more depth later, and raises important questions about the nature and biology of both autism and fragile X syndrome.

Molecular genetics of fragile X syndrome

In the majority of cases the cause of fragile X syndrome is an expansion of a CGG (cytosine, guanine, guanine) trinucleotide repeat to more than 200 repeats in the promoter region of the fragile X mental retardation 1 (FMR1) gene, located on the long arm of the X chromosome at Xq27.3. This expansion renders the region liable to methylation and consequent silencing of the gene and loss of its product, fragile X mental retardation 1 protein (FMRP), an important post-synaptic regulatory protein. Mutations in either the promoter or coding regions of FMR1 have also been reported in individuals with features of FXS, however, these are relatively uncommon (Coffee et al., 2008; Collins et al., 2010; Lugenbeel, Peier, Carson, Chudley, & Nelson, 1995; Luo et al., 2015).

The pattern of inheritance seen in the CGG expansion of FMR1 is an X-linked dominant pattern, with anticipation of symptomatology over generations as the CGG repeat sequence expands. In health, most adults have 5-54 CGG triplet repeats in this region. These repeats are generally stable in number and not liable to expansion into longer repeats over generations. However, in those with 55 to 200 repeats (known as the premutation) there is an increased likelihood of expansion during maternal meiosis into the longer repeat of more than 200 repeats necessary to silence the gene and the consequent loss of FMRP. Factors including specific repeat length and, to a lesser degree, AGG interruptions of the CGG sequence moderate the risk of expansion, however, the chance of expansion rises quickly from 55 upwards (Toledano-Alhadef et

al., 2001). Repeat lengths of 45-54 repeats are commonly reported as “gray zone” or intermediate alleles with a higher than usual chance of expansion but substantially lower than at 55 and above. Whilst intermediate alleles are more likely to expand into premutation-range expansions, they are themselves, very unlikely to undergo, in one generation, an expansion into a full mutation, although have been reported over as little as two generations (Terracciano et al., 2004).

Although the syndrome is thought to arise from the lack of FMRP, there is some variability in the levels of both FMR1 mRNA and FMRP in individuals with FXS. This adds to the complexity of the presentation. In males with FXS this may be due to mosaicism of the expansion, methylation mosaicism or other, as yet unknown, reasons. In females, the fact that differing proportions of affected X chromosomes will be randomly inactivated contributes further to this, explaining the wider range of phenotypic presentations in females with the syndrome. However, in general, IQ and FMRP largely correlate and thus IQ is not an entirely unreasonable proxy for underlying levels of FMRP (P. J. Hagerman et al., 2019).

Effect of the FMR1 premutation

Historically, carriage of the FMR1 premutation was not thought to be associated with any morbidity. However, more recently it has become apparent that it brings with it the increased likelihood of a number of conditions; notably including fragile X-associated premature ovarian insufficiency (FXPOI), fragile X-associated tremor & ataxia syndrome (FXTAS) and higher rates of mood & anxiety disorders.

Fragile X Premature Ovarian Insufficiency (FXPOI) affects approximately 20% of female premutation carriers (Sherman, 2000), with increased infertility, and menopause occurring on average 5 years early (A. Murray, Ennis, MacSwiney, Webb, & Morton, 2000). A small proportion of women will experience the menopause at a much earlier stage in their 20s or 30s. Notably, women with the full mutation do not, however, experience FXPOI.

Approximately 45% of men and up to 16% of women carrying an FMR1 premutation develop Fragile X-associated Tremor-Ataxia Syndrome (FXTAS). This neurodegenerative condition usually occurs over the age of 50 with risk of manifestation and severity increasing with age. Apart from the core symptoms of action tremor and/or ataxia (gait difficulties and disturbed limb coordination in particular) it may present with mild parkinsonism, cognitive decline (short-term memory and executive function deficits), neuropathy, neuropathic pain and autonomic dysfunction. Regarding the treatment of FXTAS, referral to neurology

should be considered, where symptomatic treatments for action tremor, parkinsonism, neuropathic pain, and mood/anxiety problems may have a role. One small trial of Memantine for cognitive effects showed no effect (Seritan et al., 2014), although there may be a role for cholinesterase inhibitors. As with the general population, treatment of contributing factors including hypothyroidism, vitamin B12 and folate deficiency and cerebrovascular risk should be considered. Long-term care of FXTAS is complex and requires a multidisciplinary approach.

Studies of premutation carriers also report a broad range of other medical problems (Campbell, Eley, McKechanie, & Stanfield, 2016; Wheeler et al., 2014), commonly including thyroid problems and mood & anxiety disorders.

Summary of fragile X syndrome and associated conditions

Whilst all driven by alterations to the same gene, fragile X syndrome and the various manifestations of the FMR1 premutation represent a broad range of phenotypes. Whilst this thesis focuses on fragile X syndrome, it is important to recognise the impact on families; not just of having a child affected by FXS, but also the wider impact of familial carriage of the FMR1 premutation. A published summary of fragile X-associated conditions based on this is included as Appendix 2 (McKechanie, Barnicoat, Trender-Gerhard, Allison, & Stanfield, 2019).

Autism spectrum disorder

Autism, or more properly autism spectrum disorder (ASD) is a phenotypic syndrome characterised by a dyad of social and communication impairments, alongside restricted and repetitive behaviours and interests. It is simultaneously a relatively heterogenous group including those with known or suspected aetiologies, as well as those for whom no known cause has been identified; and a homogenous group by virtue of the individuals sharing the features requisite for a diagnosis.

History of Autism Spectrum Disorder

Whilst there are a number of historical descriptions that have been retrospectively considered to be of individuals who may now thought to be autistic (Ireland, 1898; Itard, 1802; Tramer, 1924), the earliest descriptions more clearly aligned with what we now consider to be autism are less than a century old. Building on earlier descriptions of those described as eccentric (Kraepelin, 1915) or schizoid (Kretschmer, 1922), the early descriptions by Ssucharewa in the 1920s of an Аутистическая установка [Autisticheskaya ustanovka] (Г.Е. Сухарева, 1925) and autistischen Einstellung (Ssucharewa, 1926, 1927) or “autistic attitude” [translations in Ssucharewa & Wolff (1996) and Simmonds & Sukhareva (2019)] in children described as having schizoid

psychopathien[2] were largely missed from the English-language literature until translation in a paper by Sula Wolff (Ssucharewa & Wolff, 1996). Whilst Hans Asperger was writing about autistische psychopathien in children (but also with reference to adults) from the late 1930s (Asperger, 1938), it was only in 1981 with Lorna Wing's publication reviewing his later publication (Asperger, 1944) explicitly suggesting the eponym Asperger's syndrome that his work became better known. Rather, it was the description of early infantile autism by Leo Kanner (1943) that largely shaped the early understanding and description of what we describe as autism.

From Kraepelin's *Verschrobenen* [eccentric] patients and Kretschmer's schizoid patients, to our current understanding and definitions of Autism Spectrum Disorder (American Psychiatric Association DSM-5 Task Force, 2013; World Health Organisation, 2018), the boundaries of what we consider to be autism have moved and been shaped over time and will doubtless continue to be shaped as our understanding changes and, hopefully, improves.

It is of relevance to the overlap with fragile X syndrome that in the autism literature there is at times a tension between ideas of a narrower, or 'purer' description and those of a much broader spectrum. Even in her early work, Ssuchharewa reflected on these difficulties, deciding to, "not here deal with

[2] Ssucharewa later revised this description, replacing it with Аутичные (патологицески замкнутые) личности or "autistic (pathologically withdrawn) personality (Г. Е. Сухарева, 1959)

those of outpatients who had less obvious manifestations… and whose diagnosis was problematical“ (Ssucharewa & Wolff, 1996). These issues are age-old; Kanner bemoaning early the tendency to

“set up a pseudodiagnostic waste basket into which an assortment of heterogenous conditions were thrown indiscriminately.” (Kanner, 1973)

Attempts to generate good prevalence data have been similarly affected, with Smalley reflecting on how the use of differing criteria affected the reported prevalence rates by more than twofold (Smalley, Asarnow, & Spence, 1988).

Diagnostic Criteria

The current diagnostic criteria within the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (2013) for autism spectrum disorder are:

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history
    1. Deficits in social-emotional reciprocity;
    2. Deficits in nonverbal communicative behaviours used for social interaction;
    3. Deficits in developing, maintaining and understanding relationships.

B. Restricted, repetitive patterns of behaviour, interests or activities as manifested by at least two of the following, currently or by history:
    1. Stereotyped or repetitive motor movements, use of objects, or speech;
    2. Insistence on sameness, inflexible adherence to routines, or ritualised patterns of verbal or nonverbal behaviour;
    3. Highly restricted, fixated interests that are abnormal in intensity or focus;
    4. Hyper- or hypo- reactivity to sensory input or unusual interest in sensory aspects of the environment.

C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life).

D. Symptoms cause clinically significant impairment in social, occupational or other important areas of current functioning.

E. These disturbances are not better explained by intellectual disability (intellectual developmental disorder), or global developmental delay.

In parallel, the World Health Organisation International Classification of Disease (ICD), 11th version (2018) has a similar, if less prescriptive, description for ASD:

"Autism spectrum disorder is characterized by persistent deficits in the ability to initiate and to sustain reciprocal social interaction and social communication, and by a range of restricted, repetitive, and inflexible patterns of behaviour and interests. The onset of the disorder occurs during the developmental period, typically in early childhood, but symptoms may not become fully manifest until later, when social demands exceed limited capacities. Deficits are sufficiently severe to cause impairment in personal, family, social, educational, occupational or other important areas of functioning and are usually a pervasive feature of the individual's functioning observable in all settings, although they may vary according to social, educational, or other context. Individuals along the spectrum exhibit a full range of intellectual functioning and language abilities."

Further, ICD-11 then goes on to sub-classify ASD according to the presence or absence of intellectual impairment and the presence, impairment or absence of functional language.

Epidemiology

Early studies of the prevalence of autism, largely relying on Kanner's description of a narrowly-defined entity, gave rise to the oft-cited '4 in 10,000' figure (Lotter, 1966; Treffert, 1970). However, as our understanding and conceptualisation of autism have developed, the reported prevalence is rising. In their recent meta-analysis, Mackay et al (Mackay et al., 2017) gave their prevalence estimate as 1.04%. Whilst there are those who would assert that the real prevalence is as high as 1 in 59 (and up to 1 in 37 boys (2.70%)), one has to consider and question the methods and criteria used (Baio et al., 2018). Indeed, these most likely represent a variety of factors such as,

> "broadening of the diagnostic concepts, diagnostic switching from other developmental disabilities to PDD, service availability, and awareness of autistic spectrum disorders in both the lay and professional public". (Mayada Elsabbagh et al., 2012)

Biological underpinnings of autism

It is important to note before going on to discuss possible aetiologies of autism, that they are not necessarily exclusive of each other; nor sufficient on their own, or in combination, to fully explain autism. Whilst the diagnosis is defined by a collection of common features, the exact combination of these will be unique in each individual; and whilst it may be possible to elucidate further areas of underpinning biology or thinking style, it is likely that this will likely only ever hold for a proportion of individuals. For example, it is possible, if not likely, that a relatively rare genetic variant associated with increased rates of autism needs to be considered against the polygenic background of common variants in any given individual.

Further, the effect of environmental and social influences will always play a significant part in the manifestation of any individual's presentation. Indeed, it is noted in the ICD-11 definition that,

"symptoms may not become fully manifest until later, when social demands exceed limited capacities." (World Health Organisation, 2018).

In a similar way, a person's social context will always be a significant mediator of their presentation; and this all before ideas of secondary and or social disability are introduced by way of societal treatment of those who are seen as different or 'other'.

Genetics of autism

Whilst a number of chromosomal and monogenic disorders are associated with elevated baseline autistic traits and an increased likelihood of the presence of autism[3], a significant contribution of genetics towards the development of autism is likely to be polygenic (Grove et al., 2019). Although, as noted above the two are not mutually exclusive.

The Q2 2019 update of the SFARI Gene database includes 1089 total scored genes and 2290 copy number variant loci. These represent genes and CNVs associated with either syndromic forms of autism, or are associated with an increased chance of the development of autism; with the degree of confidence in the finding varying from negligible to high (SFARI, 2019). In their analysis of the polygenic contribution to ASD, Grove et al (2019) conclude that the contribution of common variants may be more important in individuals without intellectual disability. This is in contrast to the rarer, more highly penetrant genes identified in SFARI Gene, which may be more important in those with co-occurring intellectual impairments. What becomes clear is that whilst autism is a cluster of common phenotypic features, there are likely very many different contributing pathways. One of the challenges that faces the research field is in whether it may be possible to find commonalities of biology that span the relative heterogeneity of likely genetic underpinnings.

[3] For example: Down syndrome, XYY syndrome, fragile X syndrome, Williams syndrome.

Psychological theories of autism

Whilst it is beyond the scope of this thesis to explore all theories in great depth, it is worthwhile considering some of the main models, before going on to look at emotion processing as the focus. For a more in-depth review of psychological theories of autism, Fletcher-Watson & Happé (2019) provides an excellent overview.

Theory of Mind

The Theory of Mind (ToM) paradigm refers to the ability of an individual to attribute mental states to themselves and others, with these mental states then offering an explanation for subsequent behaviour. Whilst the term was coined in the late 1970s, the theory came to prominence in the autism literature following research using a false belief task (the Sally-Anne task[4]) by Baron-Cohen et al (1985). In their paper, Baron-Cohen et al suggested that the "dysfunction…is…specific to autism", although in this early paper, and most subsequent papers using the same task, a proportion of typically-developing children fail the task, and a proportion of autistic individuals pass the task; challenging the specificity and universality of the task, and indeed the theory itself. As such, the original, relatively simplistic ToM theory can perhaps be

[4] The Sally-Anne task is a task involving two fictional characters, Sally and Anne, usually told with puppets. Sally puts her ball in a basket, before Anne removes the ball, whilst Sally is away, and places it in a box. Upon Sally's return, the participant is asked to identify where Sally will look for the ball. The task aims to test whether the participant is able to take on Sally's perspective; that she thinks it should still be in the basket (even though the participant knows it to no longer be there).

considered as a finding which may offer some insights, but which, alone, is neither necessary, nor sufficient to explain the complexity of autism.

Executive (Dys)function

Executive function refers broadly to a range of cognitive functions which provide 'executive' oversight of lower cognitive functions and include areas such as planning, initiation, attentional control, impulse control and working memory (Chan, Shum, Toulopoulou, & Chen, 2008). Individuals with autism have been shown to have difficulties in many of these areas (Demetriou et al., 2018; Hill, 2004), although as with theory of mind, whether a theory such as this can explain the breadth of autism is not clear. Nonetheless, these broad areas of relative difficulty can provide insights into areas that may benefit from support, or where understanding of some of these phenomena may be a starting point for non-autistic individuals to start to understand autistic individuals.

Social theories

Given the primacy of impairments in social interaction and communication in the diagnostic rubrics of autism, a number of theories centre on social phenomena. The main social theory is the social orienting theory, whereby individuals with autism have been reported to orient preferentially to non-social versus social stimuli (Chawarska, Volkmar, & Klin, 2010; Dawson, Meltzoff,

Osterling, Rinaldi, & Brown, 1998; Klin, Jones, Schultz, Volkmar, & Cohen, 2002). As with previous theories discussed these differences have not been universally found (M. Elsabbagh et al., 2013) and it may be that while a general trend towards lower preference for social stimuli exists, that the significant variability between individuals means the particular paradigm used will tap into this to a greater or lesser degree. The risk of laying too much weight on a social orienting theory is that autistic individuals may be considered to have lower global orienting to social stimuli; whereas if individuals with autism are instead considered to have a different profile of social orienting (differential orienting depending on stimulus, context, other sensory cues) this may better explain the previous results. An alternative, slightly different theory, the social motivation theory, focuses more on the degree to which autistic individuals find social information innately rewarding, or not, and that diminished inherent reward from social stimuli may be central to autism (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012). However, as with the social orienting theory, it potentially attempts to overreach in what it can explain, and very poorly explains where autistic individuals are highly social, albeit in ways that are not socially typical.

One theory that is perhaps a good model for seeing how different thinking styles may lead to consequent social disability is the double empathy problem (Milton, 2012). This theory is based on the observation that any social encounter has at least two participants, and that both or all participants are actors in the interaction. Thus, any abnormality in the interaction may not /

should not automatically be attributed to the autistic actor, but rather should be considered as a problem lying with both actors and that responsibility for solving this problem should be a joint responsibility.

Weak central coherence

The weak central coherence theory posits that individuals with autism demonstrate a strong attention to local detail but diminished attention to the global overview (Frith & Happe, 1994). It suggests that in neurotypical individuals there is a strong drive to create coherence from all the available information and that the differential strengths and weaknesses of those with autism represent a diminishment of this 'central coherence'. The theory originally considered this as a particular deficit in autism, although this is perhaps better reframed as a cognitive bias towards detail in autistic individuals (Happe & Frith, 2006).

Emotion processing in autism

One of the central features of autism is a difference in reciprocal social communication and interaction. What underlies this from a biological basis has been the basis of a number of investigations (Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007; Pelphrey & Carter, 2008). Whilst this likely varies across the various aetiologies of ASD; given the clustering of features that define autism we may expect some shared underlying biology. One possible contributing factor is a differential perception of facial emotional stimuli in autistic individuals, which may then contribute to differences in social understanding, communication and interaction. Whilst the majority of studies show diminished facial emotion recognition in autistic individuals, there is significant variability in the findings; with heterogeneity in study paradigms likely to explain at least part of this (Harms, Martin, & Wallace, 2010). The time it takes for emotion recognition may also be an important difference (Clark, Winkielman, & McIntosh, 2008), with individuals with ASD typically taking longer to recognise the emotion (Teunisse & de Gelder, 2001). It should also be noted that the direction of the relationship between facial emotion recognition and the impairments in social interaction typical of autism is not entirely clear:- diminished social interaction is likely to give less exposure to facial stimuli and therefore interfere with development of the associated neural circuitry; whilst primary difficulties in facial emotion recognition may then make social interaction difficult (Leppanen & Nelson, 2006).

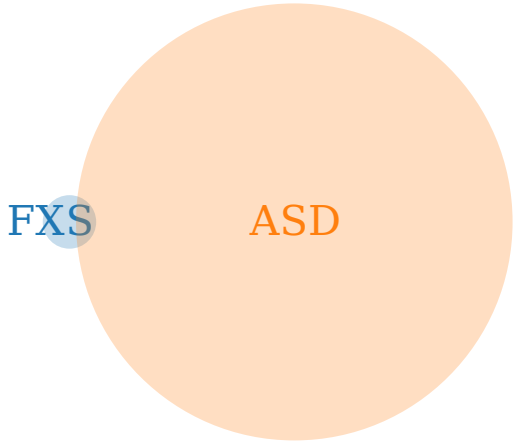
Meta-analyses of emotion processing functional imaging studies in autism show recruitment of different brain regions during facial emotion recognition, with regions of both hypo- and hyper-activation seen (Di Martino et al., 2009; R. C. Philip et al., 2012). Of these, the strongest and most consistent findings have been differences in activation in the fusiform face area (FFA) and temporal structures. In the FFA, typically hypoactivation is seen in individuals with ASD (Humphreys, Hasson, Avidan, Minshew, & Behrmann, 2008; Pelphrey, Morris, McCarthy, & Labar, 2007; Perlman, Hudac, Pegors, Minshew, & Pelphrey, 2011; Pierce, Muller, Ambrose, Allen, & Courchesne, 2001; Scherf, Luna, Minshew, & Behrmann, 2010). In their review of studies reporting on FFA activation, Perlman reports that this FFA hypoactivation was seen in 2/3^{rds} of studies, with equal activation seen in the remainder (Perlman et al., 2011). With regards to the results in temporal structures, both hypo- and hyper-activation have been reported (Ashwin et al., 2007; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007; Humphreys et al., 2008; Pelphrey et al., 2007; R. C. Philip et al., 2012), largely with a focus on the superior temporal gyrus and the superior temporal sulcus.

The intersection of fragile X syndrome and autism spectrum disorder

Whereas fragile X syndrome is a phenotypic syndrome, albeit with some variation in presentation, associated with a clear genetic cause; autism is a syndrome with a wide variety of aetiologies, both known and unknown. Thus their overlap or intersection can be conceptualised in a number of different ways. As entities, they are categorically different, a theme previously investigated and discussed by Hall et al (2010). Of note, is that discussion about whether autism in FXS is the same as idiopathic autism, is paralleled beyond FXS, with the validity of the idea of autism across the spectrum representing the same entity being questioned (Mayada Elsabbagh et al., 2012), particularly in the light of the revisions on ASD incorporated into DSM-5. Notwithstanding this issue, given the increased co-occurrence of autism in FXS the question of the nature of the overlap remains of interest. As touched on earlier, fragile X syndrome perhaps explains 0.5% of idiopathic autism, whereas perhaps in the region of 1/3rd of individuals with FXS have autism. This gives population estimates of approximately 1 in 18-20,000 individuals having ASD associated with FXS; Figure 1 visualising the extent of this overlap.

Figure 1

Relative proportions of those with FXS with ASD and correspondingly those with ASD attributed to FXS.



Reiss and Freund in 1990, in a relatively small study, described a group of 17 individuals with FXS, of whom 3 met full criteria for a DSM-III-R autistic disorder diagnosis, but a further 7 who met diagnoses for a broader pervasive developmental disorder, not other wise specified (PDD-NOS), diagnosis (Reiss & Freund, 1990). In the discussion, Reiss and Freund discuss how through different lenses, the phenotype of FXS can be interpreted as being autistic or not. Part of it pivots on the degree to which behaviours can be seen to be in keeping with developmental stage, but also the varying emphasis that may be given by diagnosticians to direct observations, parental reports and reports from other social environments, e.g. school or college.

Whilst at some level, much has changed in our understanding of these issues; at the same time little has changed. Not only is there variation across diagnostic practices internationally, but also in some countries the gating of services by diagnosis, especially so an autism diagnosis, may inadvertently influence diagnostic practice as clinicians strive to allow their patients to access appropriate support, sometimes only permitted with a certain diagnostic label. Whilst this clearly shouldn't be the case, this reality should be acknowledged during these considerations. Even in 1965, Kanner was critical of colleagues he viewed as wishing to throw,

"all curiosity about diagnostic criteria to the winds as irrelevant impediments on the road to therapy…" (Kanner, 1973)

This remark arises in the context of a discussion about how diagnostic categories are being ignored or left out altogether, so that individuals can access a given therapy that promises to be a panacea, and ignoring how a clear diagnosis may better direct the necessary treatment. Clearly, it still chimes with elements of present-day practice.

In their review of the nature of the overlap between autism and fragile X syndrome, Cornish, Turk and Levitas (2007) start to disentangle how some of the shared phenotypic presentation may reflect different developmental pathways. Whilst there are many shared features and certain overlaps in many individuals, with others most certainly meeting diagnostic criteria for both, the authors argue that a simplistic interpretation is problematic and that in many

cases the phenotype of individuals with FXS should be seen as distinct. Table 1 below (reproduced from their paper) highlights how various phenotypic aspects may reflect very different underpinnings. In many of the aspects considered, individuals with FXS have a more specific phenotype, whereas, in general, more variability is seen in the individuals with ASD; as might be expected given the heterogeneity of aetiology amongst those with ASD.

Table 1

Typical social/developmental functioning in fragile X syndrome & autism

Fragile X syndrome	Autism
Social anxiety	Social indifference
Gaze aversion	Gaze indifference
Self-injury usually in form of hand biting in response to anxiety & excitement	Self injury variable in topography & causation
Delayed imitative & symbolic play	Permanently distorted imitative & symbolic play
Hand flapping in response to anxiety & excitement extremely common	Stereotypical & manneristic behaviours highly variable in topography & causation
Language impairments characteristically comprise delayed echolalia with repetitive, rapid & cluttered speech	Language impairments highly variable, usually affecting comprehension more than expressive language
Good understanding of facial expression	Lack of understanding of facial expression
Theory of mind may be distorted but is not absent	Absent theory of mind
Characteristically friendly & sociable, albeit often shy & socially anxious with primarily communicatory & stereotypic "autistic-like" disturbances	"Aloof", "passive", "active & odd" or "overpedantic & pseudomature" with primarily social & symbolic "autistic-like" disturbances

Note. Adapted from Cornish, Turk & Levitas (2007).

What is interesting is that although Cornish *et al* highlight how the typical features of FXS and ASD may represent different developmental pathways; it is likely still the case that there is a group of individuals with FXS who do have a more narrowly-defined ASD. This likely explains at least some of the variability in the reported comorbidity between the two entities.

Conclusions

In autism we see a common phenotype with multiple possible aetiologies; whereas in fragile X syndrome we see a common genetic origin, with multiple possible clinical manifestations, both in nature and degree. In both the autism and fragile X syndrome literature, there exist a number of theories that attempt to explain both the clinical phenotype we see, but also the secondary disabilities caused by social context. Whilst the aetiology may vary widely, there remain outstanding questions as to the degree to which there are commonalities of biology that underlie the common phenotype of autism, especially across the range of cognitive ability.

Brain imaging investigation of emotion processing differences in autism and in fragile X syndrome have been commonly used to try and understand the underlying biological pathways that may underlie the observed social communication and interaction deficits/differences in these conditions. What is needed is an attempt to consider whether there are parallels in biology across a variety of subgroups, and especially across groups of differing cognitive ability.

Previous studies of functional imaging in ASD and FXS will be reviewed in more depth in chapters 2 and 3, with experimental investigations of the association of autism and emotion processing in individuals with special educational needs in chapter 5, and fragile X syndrome in chapter 6. In so doing, there is an attempt to consider whether the findings in autistic participants in either an idiopathic

intellectual impairment group, or a genetically-determined (FXS) group parallel those in the wider autism and fragile X literature.

Chapter 2: Systematic review of functional brain imaging of autism associated with intellectual impairment

Introduction

This chapter first covers the basics of magnetic resonance imaging (MRI) and functional MRI (fMRI). It then goes on to explore some of the overarching issues in the brain imaging of neurodevelopmental conditions, in particular issues arising from selection bias in recruitment of the studies and the potential benefits and pitfalls of a disease model of research. Finally, there is a systematic review of functional brain imaging studies in autism associated with intellectual impairment.

The basis of MRI methodology

Magnetic resonance imaging is a non-invasive imaging technique which uses the differential behaviour of atomic nuclei in the presence of magnetic fields to produce 2- and 3-dimensional images of materials and tissues, and in this case to allow the imaging of brain structure and function.

MRI has its origins in the 1946, with Felix Bloch and Edward Purcell independently discovering the phenomenon of magnetic resonance (Bloch, 1946; Purcell, Torrey, & Pound, 1946). The technique was principally used for chemical and physical analysis of materials until 1971 when Raymond Damadian demonstrated differential nuclear relaxation times between normal tissue and malignant tumours, proposing its utility as a medical diagnostic tool (Damadian, 1971). Further developments in the technology led to the first live animal MRI in 1976 (Damadian, Minkoff, Goldsmith, Stanford, & Koutcher,

1976), and the first live human MRI in 1977 (Damadian, Goldsmith, & Minkoff, 1977). In the intervening four decades, the technique has developed to be a commonly used medical investigation, both in clinical practice and in research.

The MRI technique is based on the principle that atomic nuclei with odd numbers of protons or neutrons (or both) exhibit a property called nuclear spin. Under the influence of a magnetic field, the spin can be induced to align with the magnetic field. The differential alignment of nuclei, either parallel or anti-parallel to the magnetic field gives rise to a net magnetisation, |M|. Upon the addition of a separate, perpendicular, oscillating radio-frequency (RF) magnetic field this alignment is disturbed; and upon the cessation of the RF magnetic field, the nuclei return to their previous state. This change in magnetisation can produce a detectable voltage in the coil of the scanner. As different tissues are made of different compositions of water (and thus with different Hydrogen ^{1}H compositions), so the tissues produce different magnetisations (and different voltages) during magnetic resonance imaging. Further coils are used to help spatially isolate virtual 'slices' for the imaging, and reconstruction of the resulting signals allows for the creation of the 2- and 3D images used for subsequent analysis.

Functional MRI methodology

Building on the basic MRI methodology, functional MRI (fMRI) offers the opportunity to observe changes in cerebral blood flow which can be used as a proxy for changes in local neuronal activity. Thus, it offers the opportunity of localising brain activity under certain conditions. When linked with stimuli or tasks presented to a scanning subject, it allows the assessment of areas of relative activity or inactivity associated with these activities.

The technique uses the observed coupling of regional cerebral blood flow (rCBF) and regional cerebral metabolic rate (rCMR) as observed in PET studies of brain activity (Yarowsky & Ingvar, 1981). By using the differential NMR signals produced by oxygenated and deoxygenated blood (by virtue of their differing neutron/proton/electron balances, and thus the presence/absence of a magnetic moment) it is possible to detect differing levels of oxygenated/deoxygenated blood at any given time – the so-called Blood Oxygen Level Dependent (BOLD) signal (Ogawa, Lee, Kay, & Tank, 1990). By considering the established link between blood flow and energy use and thinking of energy use as a proxy for brain activity, the fMRI technique allows the indirect measurement of brain activity.

As can be seen, the technique is built on a series of assumptions and the measurements made are only proxies for what is supposed to underlie and drive the measurements. Indeed each of the assumptions are subject to

challenge and inevitably the relationships between physiology and the measurements made are complex. For example, the relative contribution of neurons and astrocytes to arteriolar blood flow and glucose consumption (Gordon, Choi, Rungta, Ellis-Davies, & MacVicar, 2008; Lundgaard et al., 2015); and the roles of oxygen consumption and glucose consumption in their relative contribution to regional cerebral blood flow (Pellerin & Magistretti, 1994) are areas of discussion. Notwithstanding these uncertainties, the technique is seen as having validity in helping to understand neurobiology.

Understanding underlying neurobiology

It should be clear that endeavours to understand the underlying biology of medical conditions are of value. For affected individuals and their families, there is inherent value in understanding why they or their relative presents with a particular sign, symptom, behaviour or complication. However, further significant value comes where understanding the underlying biology can drive the development of targeted treatments; where the identification of a receptor, a pathway or a network that is dysregulated in any given condition offers the opportunity for novel treatments to be designed to regulate, modulate, amplify or inhibit.

One of the problems in the fields of neurodevelopmental disorder brain imaging research is that very commonly for pragmatic reasons, the groups who are most able to participate in the research are not necessarily representative of the

group as a whole. Indeed, as shall be explored further on in this chapter there is good evidence that large portions of those affected, and arguably most prototypical of the conditions of interest, are actually systematically excluded from the research. Thus, there is an imperative to: a) conduct more research in the under-researched areas, to help provide balance to the evidence base; b) attempt to replicate some of the findings in these un-researched groups; and c) to describe examples of how to best include these groups in future research programs.

The problems of heterogeneity in neurodevelopmental conditions

Within highly heterogeneous groups such as those with intellectual disability or those with ASD, one of the issues that arises is that of understanding how any research in one (usually highly selected, homogeneous) sample may be applicable to the wider population as a whole. This would be less of an issue if, within the broader body of research, all sub-groups were represented. Such a 'micro-segmentation' of a spectrum would allow clinicians, educators and policy-makers to select the evidence most relevant to their particular group (Mackay et al., 2017). However, as shall be explored certain sub-groups of these populations are systematically excluded and thus the evidence base, and the chances of relevant findings which may benefit them, are significantly reduced. In many cases, the samples that are included in research are very poor proxies for the populations they ostensibly represent. As has been noted,

"Studying only Kanner's syndrome or some other subgroup … will lead to conclusions of limited generalizability". (Wing & Gould (1979))

Disease models

There is good scientific reason to be narrow with regards to inclusion criteria for research; by reducing variability in the experimental and control groups, there is a higher chance of detecting a difference. Thus, what is seen in the field of neurodevelopmental research is a flourishing of research in genetically-defined conditions, so as to investigate possible neurobiological aetiologies and possible therapeutics. There is also the idea that such narrowly genetically-defined conditions, such as fragile X syndrome, which may be associated with a wider condition such as ASD, may be considered a disease model for the wider condition. Where this is of particular importance is in the development of therapeutics. Where trials in autism have failed to demonstrate efficacy, heterogeneity has often been cited as a contributory factor; often with post hoc sub-group analysis suggesting positive effect in one group or another. Thus, potentially effective medicines may be overlooked as they do not progress in their developmental programme. Whilst phase II trials are mainly focused on safety, tolerability and feasibility; the lack of strong efficacy findings understandably does not encourage neuroscience boards of pharmaceutical companies to continue the developmental pipeline. Thus disease models offer the opportunity to try and demonstrate effectiveness in narrowly-defined groups, whilst potentially offering a bridge to therapeutics to wider groups.

Difficulties of the disease model paradigm

Whilst the idea of such disease models is of clear commercial appeal to the interests of those involved in the development of medicines, it is not without problem. The fictional scenario where a treatment is found to be effective in a trial for one genetic condition associated with post-synaptic hyper-excitability and epilepsy, and is subsequently licensed for those with a similar pattern of epilepsy is arguably very different from the scenario where a medication targeting a receptor implicated in fragile X syndrome, is licensed for autism on the basis that a proportion of those with FXS also have autism. This is not to say, of course, that we should completely discount the validity of disease models as a concept, but rather that caution is needed, especially when considering how generalisable findings are.

Disease model vs. pragmatic recruitment

What can be seen is that within the body of research, there are broadly two (sometimes overlapping or nested) approaches at play: firstly a pragmatic approach to study recruitment which systematically under-represents portions of the population; and secondly, a disease model approach whereby a narrowly- (and sometimes genetically-) defined group is studied so as to help inform and potentially support use of novel therapeutics for the broader group. This disconnect poses challenges for the premise of so-called translational medicine, with silos of research in narrowly-defined groups offering the appeal of translation to larger groups, yet having limited evidence to support that. The

fact that many individuals are frequently excluded from research or trials is an issue not only for helping to understand them, but also for the field of research in general. As will be discussed further on, even within research among genetically-defined groups the issue of pragmatic recruitment often leads to a significant selection bias itself.

Review of functional MRI in autistic individuals with intellectual impairments

Despite the fact that a significant proportion of autistic individuals have an intellectual disability, very few of the studies conducted into autism either include, or focus on, these individuals. As discussed earlier, reports of the proportion of autistic individuals who have an intellectual impairment or disability varies widely; however, a recent report from the Centers for Disease Control and Prevention in the United States reported that among children with ASD, 44% were within 1 s.d. of the mean (IQ ≥ 85), 25% were in the range of 1-2 s.d. below the mean (IQ 70-84), and 31% had in IQ in the intellectual disability range (Baio et al., 2018). When looking at the fMRI literature in ASD, Philip (2012) reported in their systematic review that of 90 identified studies, only 5 studies, or 5.5%, (Gervais et al., 2004; Öktem, Diren, Karaagaoglu, & Anlar, 2001; Pierce, Haist, Sedaghat, & Courchesne, 2004; Pierce et al., 2001; Wicker et al., 2008) included individuals with a mean IQ in the range of 1-2 s.d. below the mean (IQ 70-84) and not one of them included individuals with a mean IQ in the intellectual disability range (IQ<70).

Updated review of functional MRI in autistic individuals with intellectual impairments

An updated review of the literature reveals that since the Philip (2012) review, little further research on individuals with lower average intellectual ability has been undertaken. A systematic title search in PubMed and Web of Science

including the operators autism OR ASD AND functional MRI OR fMRI resulted in 88 papers in peer-reviewed journals (16 conference abstracts published in journals were excluded). Of these 88 papers, 11 were excluded as being classification methodologies (Aghdam, Sharifi, & Pedram, 2018; Dekhil et al., 2018; Dvornek, Ventola, Pelphrey, & Duncan, 2017; Hui, Chen, & Hsieh, 2012; Mahanand, Vigneshwaran, Suresh, & Sundararajan, 2016; Nielsen et al., 2013; Sadeghi et al., 2017; Sen, Borle, Greiner, & Brown, 2018; Syed, Yang, Hu, & Deshpande, 2017; H. Wang, Chen, & Fushing, 2012; Zhao, Zhang, Rekik, An, & Shen, 2018); 9 were excluded as reviews or meta-analyses of papers published elsewhere (Aoki, Cortese, & Tansella, 2015; Bara, Ciaramidaro, Walter, & Adenzato, 2011; Buxbaum & Hof, 2013; Kana et al., 2011; Muller et al., 2011; Okamoto & Kosaka, 2018; R. C. Philip et al., 2012; Pierce, 2011; J. Yang & Hofmann, 2016); 4 were excluded as they did not have an ASD group (Ahmed & Vander Wyk, 2013; Jung et al., 2015; Mikita et al., 2016; Wilson et al., 2013); 3 were excluded as other methodological papers (Cox, Virues-Ortega, Julio, & Martin, 2017; Knaus et al., 2010; Ren et al., 2016); 3 were excluded as case studies (Dichter et al., 2010; Hugdahl, Beyer, Brix, & Ersland, 2012; Prontera et al., 2014); 2 were excluded as there was no control group (Andari, Richard, Leboyer, & Sirigu, 2016; Cociu et al., 2018); 1 was excluded as being an intervention study (Chung, Han, Shin, & Renshaw, 2016); 1 was excluded as a diffusion tensor imaging study (Q. Yang et al., 2018a); and 1 was excluded as a dataset with no analyses (W. Yan, D. Rangaprakash, & G. Deshpande, 2018). Of the remaining 54 papers, only one paper (or 1.9%)

included a group or sub-group with a mean IQ of <85 (Gabrielsen et al., 2018). Gabrielsen also identified that this is a poorly researched area, only referencing one other study which has a low-functioning group (Reiter et al., 2018). The seven papers (5 from Philip et al (2012), plus the two identified here) including individuals with ASD and a group or sub-group with a mean IQ of <85 are reviewed in detail later. Whilst this review could have been broader in its scope (for example using a broader abstract or MeSH free text search), it gives a good impression of the current state of the literature in this area. The 54 papers reviewed, along with their ASD group characteristics and fMRI paradigm details are in Appendix 3.

Other reviews of social tasks and face processing fMRI studies in autism

Of note, other recent reviews of fMRI in autism similarly reveal that the overwhelming majority of studies do not include those with lower cognitive ability. Clements et al (2018) review of social motivation in autism as measured by fMRI included 13 papers of which none had a group mean IQ of less than 100; and 10 of the 13 papers had group mean IQ of greater than 110. Similarly, in their comprehensive review and meta-analysis of face processing in autism, Aoki et al (2015) identified 13 papers, of which only one, previously identified, (Wicker et al (2008)) considered a group with a mean IQ of less than 85 (mean IQ 81.8). Whilst it is noted that fMRI studies often only include those of average, or above average, ability (Hull et al., 2016) and further suggested that, “This may be an inherent issue in fMRI studies that require the capacity to

understand the instructions related to a cognitive task in an MRI scanner, and to execute the instructions," (Dickstein et al., 2013); it is noteworthy that in other systematic reviews of cognitive aspects of autism using other techniques (EEG/MEG/ERP/pupillary response/reaction time/choice/accuracy/effort) that the groups are similarly of average or above-average cognitive ability (Bottini, 2018; O'Reilly, Lewis, & Elsabbagh, 2017). Thus, whilst there are clearly issues with fMRI that act as barriers to participation for those with an intellectual disability; many of these same barriers appear to be at play in studies using other modalities.

Review of existing literature including low-functioning autism groups.

Table 2 sets out the 7 studies identified earlier as having groups with low mean IQ. Of these papers, 6 examine groups with mean IQ in the range of 1-2 s.d. below the mean (70-84) and 1 examined a group in the intellectual disability range (Gabrielsen et al., 2018). By comparison, the mean IQ of the comparison groups was at least 102.34 (only 2 of the 7 reported comparison group IQs, and thus it is considered that this is likely an underestimate of the true value). Of the total of 79 participants there were 62 males and 8 females, plus 9 participants of unknown sex. The ages of participants ranged from 7 to 53, with the three studies in childhood (Gabrielsen et al., 2018; Öktem et al., 2001; Reiter et al., 2018) having narrower age ranges and lower group average IQ. Of the seven studies, five report data from traditional task-based analyses (Gervais et al., 2004; Öktem et al., 2001; Pierce et al., 2004; Pierce et al., 2001; Wicker

et al., 2008), two (Gabrielsen et al., 2018; Reiter et al., 2018) use a functional connectivity approach and one reports an effective connectivity analysis (Wicker et al., 2008).

Table 2
Summarised results from review of functional imaging in ASD and intellectual impairment.

Author (year)	Autism group					Comparison group(s)				Control matching criteria
	N (M:F)	Mean age (S.D., range)	Mean IQ (S.D., range)	Diagnosis	ASD features	Type of Group	Mean age (S.D., range)	Mean IQ (S.D., range)	Autism features	
Gervais (2004) *	5:0	25.8 (5.9, 21 – 34)	81 (18.8)	Autism	DSM-IV & ADI diagnosis of autism.	HC control group 8 males	27.1 (2.9, 21-31)	Not noted	Not noted	Age, gender
Oktem (2001) *	9 (sex not noted)	12 (3.1, 7-17)	76.8	AS	DSM-IV diagnosis of Asperger's Syndrome	Paediatric neurology outpatient controls 8 (sex not noted)	11.0 (2.5, 8-16)	Noted as normal range	Not noted	Age, handedness
Pierce (2001) *	7:0	29.5 (8, 21-41)	83.7 (10.9)	Autism	CARS Mean (S.D., range) 34 (3.4, 30-39)	NT control group 8 (sex not noted)	28.3 (20-42)	Not noted	Not noted	Age, gender, handedness, task performance
Pierce (2004) *	7:0	27.1 (9.2, 16-42)	80.3 (17.7)	Autism	ADOS Communication+ social total) Mean	NT control group 7 males	16-40	Not noted	Not noted	Age, gender, handedness, task performance

					(S.D., range) 16.63 (12-20)					
Wicker (2008) *	11:1	27 (11, 18-53)	81.3	8 Autism, 4 AS	DSM-IV	HC control group 14 males	23.4 (10)	Assessed but not reported.	Not noted	Age,
Gabrielsen (2018)	LVCP 14:3 HVCP 15:5	12.26 (3.34, 7-17) 12.64 (2.87, 7-17)	54.00 (17.50) 106.85 (13.64)	ASD	ADOS-2 CSS LVCP 7.94 (1.52, 4-10) HVCP 7.35 (.01, 4-10)	NT control group 14 males, 5 females	11.76 (2.61, 7-17)	111.76 (13.05, 85-134)	Not noted	Matched (no detail)
Reiter (2018)	L-ASD 18:4 H-ASD 19:3	L-ASD 11.1 (2.7, 7-15) H-ASD 11.1 (2.8, 7-15)	L-ASD 77 (6, 61-85) H-ASD 123 (8, 106-138)	ASD	ADOS-2, 14 (5, 5-24) 11 (4, 6-21)	Average FSIQ typically-developing control group 15 males, 7 females Higher FSIQ typically-developing	11.0 (2.8,6-15) 10.8 (2.0, 8-14)	99 (7, 88-112) 124 (8, 108-144)	Not noted	Head motion, handedness, and age

						control group 17 males, 5 females				
Totals (only including L-ASD & LVCP groups from Gabrielsen and Reiter)	79 total 62 male 8 female 9 unknown (Sex not noted by Öktem)	17.85 (9.79)	74.2 (17.33) SD of 15 assumed for Öktem and Wicker			86 total 58 male 12 female 16 unknown	17.68 (9.1) Missing mean and s.d. figures imputed to give above figure.	102.34 (13.4) However, figures only given for 2/7 studies. Mean (s.d.) of 100 (15) assumed for missing data. Likely underestimate.		

Note. * included in Philip et al (2012)
ADI, Autism Diagnostic Interview; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; CARS, Childhood Autism Rating Scale; DSM-IV, Diagnostic and Statistical Manual of the American Psychiatric Association, Fourth Edition; FSIQ, full-scale IQ; H-ASD, higher cognitive ability ASD; HC, healthy control; HVCP, higher verbal and cognitive performance; L-ASD, lower cognitive ability ASD; LVCP, lower verbal and cognitive performance; NT, neurotypical.

Task-based studies

Social judgement task

In their study of children with Asperger's syndrome, Öktem et al (2001) examined fMRI signal intensity during a social judgement task. The task involved asking the children to make a social judgement on a fictional scenario; to think about it during a 1-minute functional MRI; and to give their answer following the scan. For those with Asperger's syndrome, they found that in 4 of 9 individuals there was no detectable change in frontal signal intensity (either increased or decreased) compared to a noted change in signal intensity (6 activation; 2 suppression) for all control participants.

Face-processing tasks

Facial processing has long been an area of interest in the autism field. As discussed in chapter 1, theories around autistic individuals' ability to understand and interpret facial expressions have been posited as at least partially explaining the relative difficulties in social communication (Adolphs, Sears, & Piven, 2001; Hobson, 1986; Pelphrey et al., 2002).

In the study by Pierce (2001) they compared seven adult males with autism to eight typically-developing controls on a task comparing the BOLD signal response to pictures of faces as opposed to pictures of equivalent-luminance shapes. They reported significantly lower volumes of activation in the autistic group in the fusiform gyrus (FG) bilaterally and the left amygdala. There were no

group differences in the other *a priori* regions-of-interest (ROIs), however, they did report greater heterogeneity in patterns of activation in the autistic group compared to the control group; "each autistic patient had a distinct region of functional activation in response to faces…in contrast to the consistent FG activation in normal subjects".

In a further study by Pierce (2004), this time examining differential response to familiar vs. unfamiliar faces, there were no significant between-group effects in the fusiform face area (FFA), amygdala or on whole-brain analyses. Prompted by differential findings in the within-group analyses, more liberal post hoc analyses showed a number of regions of greater functional activity in the control group in response to familiar faces, stranger faces and familiar versus stranger faces; although given the lower thresholds used, the importance of these results is not clear.

Although their main focus was on effective connectivity, Wicker et al (2008) reported data on BOLD signal differences between a group of 12 individuals with ASD and a comparison group of 12 controls (mean IQ 81.8). In their whole-brain comparison of brain activation to explicit emotion recognition between the groups, 3 areas showed significantly greater activity in the control group compared to the ASD group associated with explicit emotion recognition (Right temporo-parietal junction, right inferior frontal gyrus Brodmann Area (BA) 45 and medial superior frontal gyrus BA 9/10).

Voice-processing task

In a similar vein to the comparison of brain activity to faces versus shapes; so the study by Gervais (2004) examined the functional brain activity response to voices versus non-voice sounds. Drawing the comparison that the superior temporal sulcus (STS) could be considered the “auditory cortex counterpart” of the FFA, the study compared a group of five male adults with autism with 8 matched controls. In it they found that the control group showed significantly greater activation in the upper bank of the STS for voice compared to non-voice stimuli, and conversely the autistic group did not show any areas significantly more activated by voices.

Connectivity studies

Functional connectivity

Whilst all of the studies reviewed previously have been event- and block-designed fMRI studies, an area of increased interest is in that of connectivity studies. Functional connectivity analyses examine the statistical correlations between remote brain regions to draw conclusions about likely connections and their relative strength. Whilst many have tried to characterise the ‘typical’ changes in connectivity in autism, a simple conclusion has remained somewhat elusive with reports of both over- and under-connectedness under different experimental conditions and with different populations. The best that can probably be concluded is that more diffuse patterns of connectivity are seen in

autism (J. V. Hull et al., 2017), with future work needing to consider carefully the various possible experimental and analytic confounds (Vasa, Mostofsky, & Ewen, 2016). Typically, studies use a so-called 'resting-state' sequence in which the participant is typically asked to remain awake, stay still and either keep their eyes open or closed. In some paradigms a fixation cross is used to concentrate on, whereas in others standardised visual stimuli have been used to hold attention, such as the 'Inscapes' movie (Vanderwal, Kelly, Eilbott, Mayes, & Castellanos, 2015). Functional connectivity studies offer the opportunity to visualise the degree of connectedness within and between various brain structures; which may be important in cases where the differences are not in activation in specific brain regions, but rather in the way and degree they interact with each other.

In their study of individuals with lower-functioning autism, Reiter (2018) compared resting-state fMRI data from 44 children with ASD to data from 44 typically developing controls. Within their ASD group, participants were divided into lower- or higher-functioning subgroups, with the lower-functioning group (n=22) having a mean IQ of 77. Their main findings as related to the lower-functioning group were of significant underconnectivity between the mPFC and the precuneus / posterior cingulate gyrus compared to the higher-functioning ASD group; and of over-connectivity between mPFC and pericalcerine cortex compared to TD controls of average cognitive ability. Notably, they describe different findings in the higher-functioning group (mainly of significantly

decreased anticorrelations among default mode network, salience and task-positive regions), raising further questions about the legitimacy of spectrum-wide inferences from individual, narrowly-recruited studies.

Gabrielsen (2018) also examined functional connectivity; comparing findings between a group of typically-developing children, a group with ASD and high verbal and cognitive performance (HVCP), and group with ASD and low verbal and cognitive performance (LVCP). Their LVCP group had a mean IQ of 54 (s.d. 17.50) compared to the HVCP group with a mean IQ of 106.85 (s.d. 13.64) and the typically-developing group with a mean IQ of 111.76 (s.d. 13.05). Whilst they report significantly lower within-network functional connectivity in the LVCP group compared to the HVCP group in four networks (auditory, default, frontoparietal and salience); in each of these networks there was no significant difference between the LVCP and typically-developing groups. This may at least be partially explained by the TD group having the highest mean IQ of all three groups and the finding that lower IQ was associated with decreased connectivity globally. Using global signal regression (GSR) to account for these differences in median connectivity, they report the same pattern in LVCP vs HVCP as LVCP vs neurotypical controls, suggesting a “signature” pattern of connectivity in autism.

Effective connectivity

Effective connectivity analysis builds on functional connectivity analyses and explicitly seeks to establish the influence of one region/system on another. Wicker et al (2008) examined 12 individuals with autism, with assessed IQ ranging from 64 to 127 (mean 81.8) and examined effective connectivity of 'social brain' structures by structured equation modeling, selecting seven brain regions of interest based on previous research. In their study, they used a block design paradigm in a 2x2 factorial design with participants being asked to report on either the emotional state of an actor or the age of the actor with the second factor being the direction of gaze of the actor (either averted or direct). In their effective connectivity modeling analyses four regions were identified as having significantly stronger or weaker connections between the groups. Controls showed significantly stronger connections than the ASD group from: dorso-medial prefrontal cortex to dorsolateral prefrontal cortex; occipital cortex to fusiform gyrus; and dorsolateral prefrontal cortex to ventorolateral prefrontal cortex; whereas the ASD group showed a stronger connection from the dorsolateral prefrontal cortex to the fusiform gyrus.

Discussion

In the studies examining the neural response to the explicitly social stimuli of faces and voices, as well as the social judgment task study, the most striking similarity in the results is that of a greater response to the given task / stimuli in the control groups being studied. These regions included the

frontal/frontoparietal regions (Öktem et al., 2001), bilateral fusiform (Pierce et al., 2001), left amygdala (Pierce et al., 2001), right temporo-parietal junction (Wicker et al., 2008), right inferior frontal gyrus (Wicker et al., 2008), medial superior frontal gyrus (Wicker et al., 2008), and left & right superior temporal sulcus (Gervais et al., 2004). Trends towards greater activity on various contrasts were also described in the right anterior cingulate, medial frontal lobe, putamen, supramarginal gyrus, caudate, precuneus inferior parietal lobe, medial frontal lobe; and the left thalamus (Pierce et al., 2004). The only area with a reported trend increase in activation in the autism group was in the right postcentral gyrus in a contrast examining response to familiar versus stranger faces (Pierce et al., 2004). In parallel, the findings in the connectivity studies showed a mixture of under- and over-connectivity in the ASD groups in functional connectivity studies (Gabrielsen et al., 2018; Reiter et al., 2018) and a similar mix of increased effective connectivity across different regions in both ASD and control groups (Wicker et al., 2008). Interestingly, Gabrielsen summarized that their findings represented increased between-network connectivity along with reduced within–network connectivity in the LVCP group, although noting that these findings were also associated with low IQ, it is difficult to draw too many conclusions. Perhaps these findings are best seen in the light of a recent review of connectivity studies in ASD more generally, where the authors concluded,

> "While hallmark connectivity patterns are still unclear, evidence suggests that ASD is most likely characterized by instances of both under- and over-connectivity." (J. V. Hull et al., 2017).

One of the persisting issues with these studies is that it is not clear to what degree IQ was a confounding variable in the analyses done. Only Reiter et al describe including it as a covariate in a subset of their analyses, whereas in most of the studies, the IQ of the control group is not even reported (Gervais et al., 2004; Öktem et al., 2001; Pierce et al., 2004; Pierce et al., 2001; Wicker et al., 2008). Without consideration of this as a factor, or the use of an IQ-matched control group, it is very difficult to assess the degree to which the findings attributed to the autism diagnosis are, in fact, accounted for to a degree by the group IQ differences. The counter-argument may run, however, that if autism itself is associated in a substantial proportion of people with intellectual impairments, then including IQ as a covariate may in fact remove some of the differences which may be accounted for by autism. On balance, however, attempts to have control groups matched on IQ, or failing that to include IQ as a covariate would help tease out what differences can be attributed to the added diagnosis of autism.

Conclusions

In the review of functional MRI studies in ASD there was a very significant selection bias in studies towards those of above average IQ. Further, in the studies identified, where the ASD group were of below average IQ, the control group remained of neurotypical subjects and IQ was usually excluded as a potential confounding variable. This has a significant bearing on the generalizability of the findings and potential implications for both our

understanding of the underlying neurobiology as well as the development of any potential therapeutics.

Chapter 3: Systematic review of functional brain imaging of functional brain imaging of fragile X syndrome

Introduction

Building on the previous chapter, this chapter is a systematic review of functional brain imaging studies of fragile X syndrome, with discussion of the domains examined and their results. There is also discussion of over-arching issues such as selection of comparison groups and the degree to which the FXS participants represent individuals with FXS more generally. The chapter concludes with a brief consideration of future directions based on both this and the review in the previous chapter.

Systematic review of functional imaging in fragile X syndrome

Review methodology

PubMed and Web of Science databases were searched for all English language studies published between January 1990 and October 2018 that reported functional MRI data in people with fragile X syndrome. The search terms included 'fragile x syndrome' OR 'FMR1' combined using the AND operator with 'imaging' OR 'MRI'. Title, keyword and free-text abstracts were used. Further, the reference lists of included articles were reviewed for further possible articles.

Inclusion criteria

Articles were only included if they were primary research studies published as peer-reviewed articles in English and they compared a sample of participants

with full-mutation fragile X syndrome with a group of controls, using fMRI. Both neurotypical and intellectual disability control groups were included. Abstracts were screened for inclusion, and full text articles were obtained where they passed this screening. The full articles were then reviewed to confirm eligibility for inclusion. The reference lists of the retrieved articles were also screened for further eligible articles.

Data extraction

For each of the included papers, data were extracted on the fragile X group including mean age, IQ and details of diagnosis. In the main, this was confirmed full-mutations of FMR1, however, some studies also reported on the inclusion of individuals who were mosaic for the full-mutation (with a proportion of cells showing the premutation). In addition, details were extracted on the comparison groups including mean age and IQ. Finally, details were recorded on the domains by which the groups were reported as being matched.

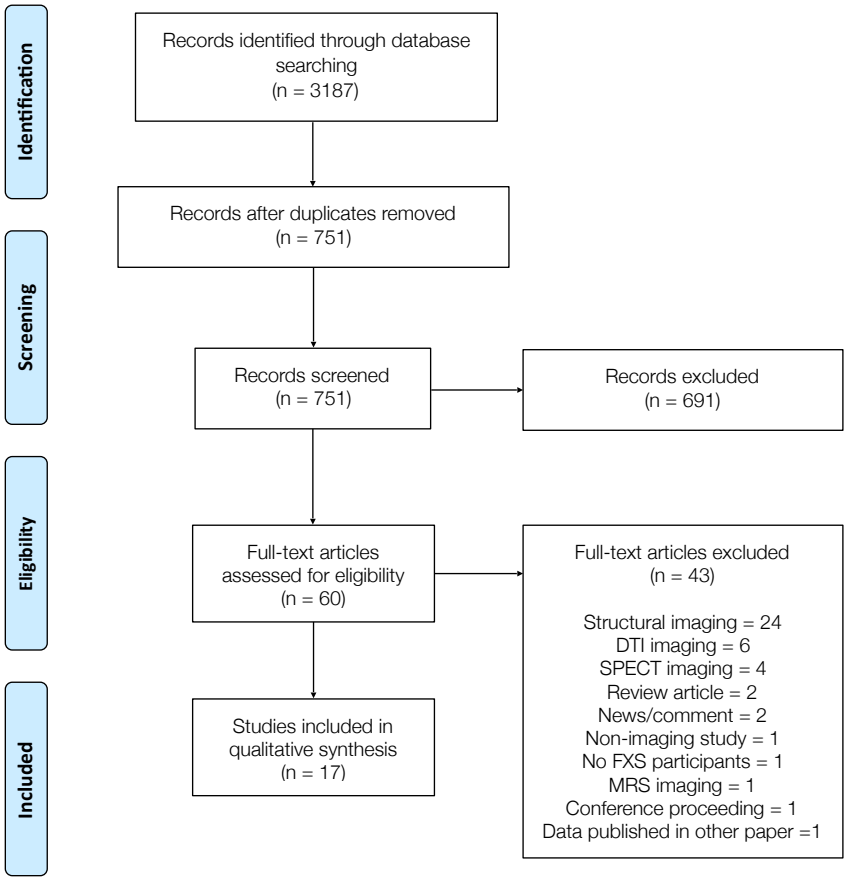
Results of the review

Included papers

Figure 2 shows the papers identified at each stage of the review, with 17 papers being finally included.

Figure 2

Flow chart of Fragile X Syndrome functional imaging research review

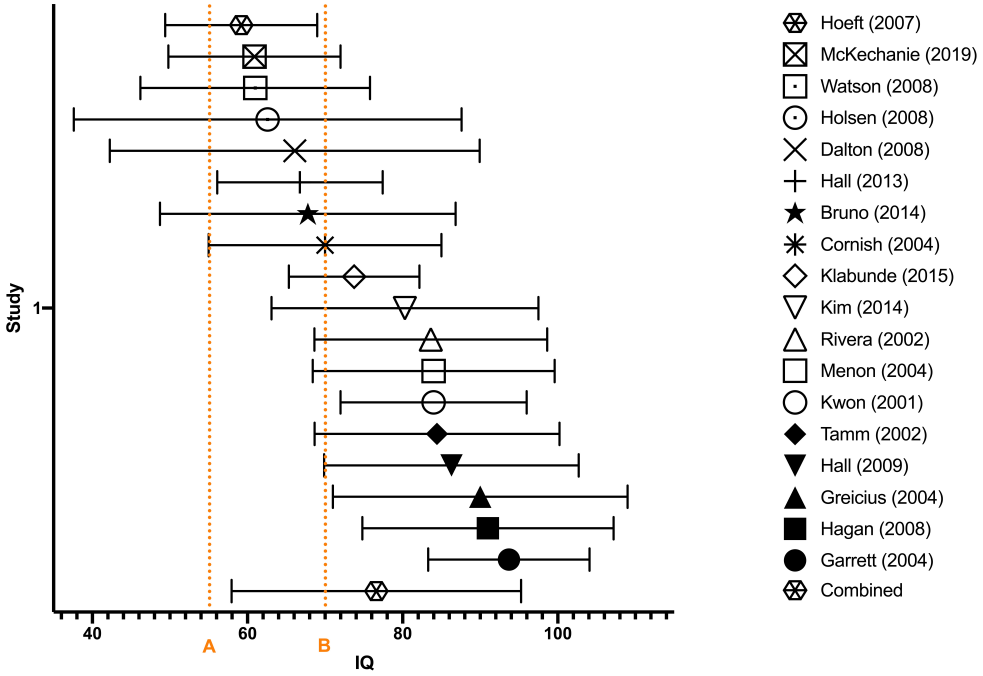


Participant details

The 17 studies included a total of 215 participants, of which 155 were women or girls, and 60 were men or boys. Of the studies, 9 studies were of women or girls with FXS, 6 related to mixed-gender groups and 2 were studies of men or boys alone. The largest study (Bruno, Garrett, Quintin, Mazaika, & Reiss, 2014) had 27 FXS participants, whilst the smallest study (K. Cornish et al., 2004) included three participants. The mean number of FXS participants per paper was 13. With regards to the mean IQ of the groups, 4 studies had a FXS group with a mean IQ of 85 or above; 7 studies with a group mean IQ of 70-84; and 6 studies with a group mean IQ of 69 or lower (range 59-68). The mean IQ of all participants across the 17 studies was 76.62, compared with a mean IQ of the comparison groups of 100.47. As with the functional MRI studies in ASD + ID, this appears to reflect an over-representation of more able participants, which may skew the findings, when taken in aggregate. Each of the studies explores and discusses these factors, and some of them consider IQ as a covariate, however there is a clear lack of research evidence in functional imaging among those who are the most impaired. Figure 3 shows the distribution of IQs among the studies included in the review, plus the study presented in chapter 6 (McKechanie, Campbell, Eley, & Stanfield, 2019) (Appendix 4) for comparison. The included papers and their group characteristics are detailed in Table 3.

Figure 3

Mean IQ of FXS participants in functional MRI imaging studies



Note. Shows the Mean IQ (whiskers denote +/- s.d.) of FXS participants in the 17 studies reviewed, plus the participants in the study presented in chapter 6 (McKechanie, Campbell, et al., 2019) (Appendix 4) and the combined total. Line A at IQ of 55 for reference as boundary of mild and moderate intellectual disability (65-85% of boys with FXS have a FSIQ <55 (Hessl et al., 2009)). Line B at IQ 70 represents the boundary for intellectual disability, with those having an IQ <70 considered to have an intellectual disability.

Table 3
Summarised results from review of functional imaging in fragile X syndrome. ASD features in comparison groups presented in same order and format as for FXS group ASD features.

	FXS group				Comparison group(s)				Control matching criteria
Author (year)	**N (M:F)**	**Mean age (sd, range)**	**Mean IQ (sd, range)**	**ASD features**	**Type of group**	**Mean age (sd, range)**	**Mean IQ (sd, range)**	**ASD features**	
Bruno (2014)	13:14	20.93 (2.75, 15-25)	67.78 (19.05, 40-119)	ADOS social + communication composite (mean (s.d., range)) 6.59 (6.08, 0-18)	IQ-matched control group 12 male, 12 female	19.00 (3.15, 15.14-25.77)	75.00 (20.73, 53-123)	6.25 (4.67, 0-16)	Sex, IQ, ASD symptoms, adaptive behaviour (VABS)
Cornish (2004)	0:3	24.3 (6.8, 19-32)	Normal-mild ID	Not noted	HC control group 12 (sex not noted)	25.9 (5.6, 19-36)	Not noted	Not noted	nil
Dalton (2008)	3:6	20.7 (2.77, 17-24)	66.1 (23.84, 35-95)	SCQ (mean (s.d., range)) – 9.9 (4.70, 1-16)	ASD control group – 14 males TD control group – 12 male, 3	15.9 (4.71, 10-25) 16.8 (2.57, 13-23)	87.2 (25.84, 35-122) Not noted	26.1 (4.27, 19-32) Not noted	nil

					female				
Garrett (2004)	0:11	16.4 (4.09, 10-22)	93.7 (10.4, 80-111)	Not noted	TD control group 11 females	15.5 (3.41, 10-22)	107.0 (11.2, 85-120)	Not noted	age
Greicius (2004)	0:12	15.2 (4.7, 7-22)	90 (19)	Presumed nil autistic as "screened for…developmental disorders)	HC control group. 16 females.	14.9 (3.8, 9-22)	113 (15)	Presumed nil autistic as "screened for…developmental disorders)	Age, sex
Hagan (2008)	0:10	16.4 (4.9, 9.7-24.0)	91 (16.2, 75-124)	Not noted	TD control group 10 females	15.6 (4.2, 8.4-22.9)	106.1 (15.7, 79-128)	Not noted	Age, sex
Hall (2009)	0:10	18.7 (3.81) Developmental age – 15.78 (2.82)	86.3 (16.41)	Not noted	TD control group 10 females	14.7 (2.95) Developmental age – 15.79 (3.32)	110.2 (10.84)	Not noted	Developmental age, sex
Hall (2013)	8:9	17.52 (4.68)	66.76 (10.67)	SCQ (mean (S.D.) 9.59 (6.52)	DD control group 12 male, 4 female	16.31 (4.06)	63.81 (11.53)	11.62 (7.47)	Age, IQ, and severity of behavioral and cognitive symptoms, SCQ

Hoeft (2007)	10:0	15.4 (2.7)	59.2 (9.8)	Not noted	TD control group 10 males DD control group 10 males	16.74 (4.2) 14.56 (2.7)	125.6 (11.5) 65.3 (13.8)	Not noted	Age and gender-matched TD Age, gender and IQ-matched DD
Holsen (2008)	5:6	18.5 (4.1)	62.6 (25.0)	SCQ (mean (S.D.) 14.6 (10.0)	TD control group 5 male, 6 female	18.7 (5.8)	120.9 (13.0)	Not noted	Age & gender
Kim (2014)	6:10	14.0 (2.97)	80.3 (17.2, 53-117)	Number meeting ADOS ASD criteria (presumed cut-off of 7 used) - 5 SRS (mean (S.D., range))- 64.5 (15.7, 39-92)	NT control group, 10 male, 10 female	13.7 (3.24, 8-17);	111.8 (17.7, 88-137)	Not noted 40.3 (5.5, 34-51)	Age
Klabunde (2015)	2:6	18.88 (4.11)	73.75 (8.41)	SCQ (mean (S.D.) 7.63 (6.05)	IQ-matched control group 8 male, 2 female	17.04 (3.49);	68.00 (13.67)	10.20 (7.38)	Age, IQ, SCQ
Kwon	0:10	17.23 (4.49,	84 (12, 65-	Not noted	TD control	15.05 (4.58,	117 (13, 93-	Not noted	Age

(2001)		10.2-22.7)	108)		group. 15 females	7.66-21.6)	137)		
Menon (2004)	0:18	15.95 (4.02)	84 (15.6)	Not noted	HC control group. 16 females.	15.5 (3.85)	117 (11.8)	Not noted	Age, sex
Rivera (2002)	0:16	16.17 (10.12-22.73)	83.63 (52-108)	Not noted	TD control group 16 females	16.97 (10.85-22.67	122.69 (99-142)	Not noted	Age, sex
Tamm (2002)	0:14	15.43 (3.79, 10-22) (for both FXS and control groups)	84.43 (15.79)	Not noted	TD control group 14 females	15.43 (3.79, 10-22) (for both FXS and control groups)	117.93 (13.21)	Not noted	Age, sex
Watson (2008)	13:0	15.5 (2.4)	61.0 (14.8)	Not noted	DD control group – 10 males TD control group – 13 males	16.1 (13.3) 15.0 (2.5)	62.4 (9.4) 116.8 (11.6)	<15 on ASQ	Age, sex, IQ (DD group)
Totals	215 total 60 male 155 female	17.23 (4.18)	76.6 (19.1)		273 total 116 male 145 female 12 unknown	16.46 (5.05)	100.47 (25.47)		

Note. ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; DD, developmental disability; NT, neurotypical; SCQ, Social Communication Questionnaire; TD, typically-developing; VABS, Vineland Adaptive Behaviour Scale.

Task domains

The 17 studies covered 4 broad domains including facial/emotion processing, auditory processing, cognitive functions (memory, attention, cognitive interference), and functional connectivity. Each domain and the studies examining it are considered below, with more time spent on the face/emotion processing studies as the focus of this thesis.

Studies of gaze/facial/emotion processing

The previous studies of face and gaze processing in fragile X syndrome have produced relatively heterogenous findings (Bruno et al., 2014; Dalton et al., 2008; Garrett et al., 2004; Hagan et al., 2008; Holsen et al., 2008; Kim et al., 2014; C. Watson et al., 2008). As with the autism literature, this has likely been the result of a combination of factors including: imaging paradigm used, balance of gender, level of intellectual functioning of the FXS group and choice of comparison group. Of particular note is that given the direct relationship between IQ and FMRP levels (P. J. Hagerman et al., 2019), it is likely that some of the variability will be explained by the wide range of group mean IQ (61-94) of the individuals in these studies, and thus the underlying FMRP levels. Further, in these 7 studies the participants included 57 females and 43 males; this representing somewhat of a selection bias for females. These participant characteristics likely reflect the significant difficulties in recruiting and scanning

individuals with more significant intellectual impairment, who are more likely to be male.

Gaze processing

In their study of 13 boys with FXS, Watson (2008) examined the brain activation associated with stimuli showing direct and averted eye gaze. In their within-group analysis they describe distinct regions of activation in each of the FXS, typically-developing control, and developmental delay control groups. In their whole-brain analysis of direct gaze > averted gaze, they further describe a main effect of group for three regions of significant activation. Extracted values for these three regions showed significantly increased activity in the FXS group in the left insula, and significantly decreased activity in right midfrontal gyrus and right cingulate. A region of interest analysis found no differences in amygdala activation, but did show a significant effect in the right STG, with post hoc tests showing significant difference in this cluster between each of the three groups; DD>FXS>TD. In further analyses they report on increased sensitisation in the left amygdala in FXS participants to successive direct gaze exposure.

In their study of facial-emotion processing, Dalton (Dalton et al., 2008) compared 9 individuals (6 female, 3 male) with fragile X syndrome against typically-developing and ASD comparison groups. The in-scanner task involved participants being asked to decide whether the face shown was emotional or neutral. Simultaneously, eye-tracking data were acquired to allow for the

participant's gaze direction in relation to the images shown to be analysed alongside the imaging data and the accuracy data of the participants decisions. In their analysis of activation differences they reported left frontal gyrus hypoactivation in FXS compared to TD controls; and increased activation in left hippocampus, right Insula, left postcentral gyrus and left superior temporal gyrus in FXS compared to TD and ASD controls. In addition they report a number of correlations between IQ, SCQ score and average eye fixation in the FXS group: IQ being negatively correlated with left hippocampal activation; SCQ being positively correlated with left hippocampal activation and left amygdala activation but negatively correlated with right fusiform gyrus activation; and average eye fixation being positively correlated with right and left fusiform gyri activation, in keeping with what might be predicted.

Bruno et al's (2014) study of face and gaze habituation is the largest of the functional imaging studies in fragile X syndrome found in the review, with 27 FXS participants scanned, compared to 25 cognitive ability and autism symptom matched controls. There was no main effect of gaze direction found and no significant group by gaze interaction. However, using the two runs of the task to consider habituation and sensitisation to the images, they report reduced habituation (and increased sensitisation) in the fragile X group in response to all faces (with both direct and averted gaze) in the cingulate gyrus, fusiform gyrus and frontal cortex compared to both the IQ- and ASD-matched controls. As might be expected they also found that ADOS score correlated

negatively and FMRP (as a %) correlated positively with habituation in the fusiform gyrus, although this finding only held true for the females in the study. For the FMRP this is, at least in part, likely because over 75% of the male participants had FMRP levels <20% and over 50% had levels <10% making a relationship more difficult to detect with the numbers present.

Garrett (2004) examined the neural systems underlying face and gaze processing in 11 females with fragile X syndrome. Interestingly, the group had a mean IQ of 94, and so the degree to which this group represents the breadth of fragile X syndrome is debatable. Nonetheless, even if we perhaps expect the power of a study with a group such as this to perhaps be reduced, there may still be important insights to be found. Firstly, there was no whole brain group difference when comparing angled faces to forward-looking faces. Secondly, when considering the neural response to averted gaze, three clusters (STS, lingual gyrus, and cerebellum) of greater activity were found in the controls versus FXS participants and two clusters (right insula and cerebellum) of greater activity were found in the FXS participants versus controls. Finally, region of interest analyses considered the fusiform gyrus and the superior temporal sulcus. In the fusiform gyrus controls had significantly greater activation to forward-looking faces than averted faces, whereas the FXS group did not show this pattern, even if the between-group analysis had not shown this at a significant level. In the superior temporal sulcus they reported that control participants had greater STS activation to all stimulus conditions combined

compared to the FXS participants.

Emotion-processing studies

Holsen (2008) aimed to examine whether the underlying neural circuitry of face encoding was disrupted in FXS, and the degree to which activation related to this was mediated by social anxiety. They studied 11 individuals with FXS (6 female, 5 male) in comparison to 11 age- and gender-matched controls. Their main finding was of decreased activation in the FXS group compared to the control group in left superior frontal gyrus and medial frontal gyrus when considering activation related to encoded faces. They also reported that different regions of activation in the control and FXS groups correlated with social anxiety measures; with significant group differences in the left angular gyrus, left insula and left posterior cingulate gyrus; suggesting a potential relationship between social anxiety and encoding of social information.

In their study of fear-specific amygdala function, Kim (2014) used fMRI to examine neural responses to fearful, happy and scrambled faces in 16 individuals (10 female, 6 male) with fragile X syndrome compared to neurotypical controls. They reported 4 clusters of significantly reduced activation in the FXS group compared to controls across fearful > happy faces (bilateral amygdala, left fusiform, bilateral anterior cingulate, and bilateral insula), fearful > scrambled faces (right ventrolateral prefrontal cortex (VLPFC), left and right striatum, bilateral amygdala, bilateral anterior cingulate, and bilateral insula) and happy > scrambled faces (right precuneus, superior/middle occipital gyrus,

and right superior temporal gyrus) contrasts in the FXS group compared to controls. Further, they reported significant positive correlations between FMR1 expression and amygdala and right anterior cingulate activation, and significant negative correlations between anxiety/social dysfunction scores, and amygdala activation in the FXS group.

Hagan's (2008) study of emotion processing in 10 females with fragile X syndrome considered the neural response during an emotion attribution task, compared to a typically-developing control group. The study reported results arising from a number of contrasts including: reduced activation in the FXS group compared to the controls on the neutral faces > scrambled faces contrast in clusters centred on the right cingulate gyrus, precuneus and bilateral dorsal anterior cingulate cortex (dACC); reduced activation in the caudate on the sad faces > scrambled faces contrast in clusters centred on bilateral lentiform nuclei, left superior frontal gyrus, left inferior parietal lobule and bilateral precunes; and reduced activation on the sad faces > neutral faces contrast in clusters centred on right cuneus, right precentral gyrus and left inferior parietal lobe. In contrast, the FXS group showed three clusters of greater activity compared to controls on the happy faces > neutral faces contrast with in clusters centred on the left lingual gyrus, right precentral gyrus and left precentral gyrus. Correlational analyses in the FXS group showed a correlation between FMRP protein levels and activation of the dACC, to all three main contrasts, with the effect surviving even after covarying for IQ.

Studies of auditory processing

Only one study by Hall et al (2009) was found in this category, investigating the neural basis of auditory temporal discrimination in FXS. The study compared 10 females with FXS against 10 typically-developing but developmental age-controlled participants on a task asking participants to discriminate between on the relative length of auditory tones. They found significantly greater activity in a number of left hemispheric structures including left medial frontal gyrus, left superior and middle temporal gyrus, left cerebellum, and left brainstem (pons) in the FXS group. There were no corresponding regions of increased activity in the control group relative to the FXS group. Correlational studies showed the cerebellar result correlated with mental age in the FXS groups. Performance on the task correlated with cerebellar activation in the TD group and medial temporal activation in the FXS group. There were no correlations with FMRP level found in the FXS group.

Studies of cognitive functions

The papers considered here cover a variety of cognitive domains and processes, with 2 domains (memory and attention/response inhibition) having more than one paper each, and the remaining three domains (equivalence relations, cognitive interference and arithmetic processing) having one paper investigating each of them.

Studies of memory

Kwon (2001) compared 10 female subjects with FXS to 15 typically-developing (TD) females on a 1-back and 2-back visuospatial working memory task with region of interest analyses on the inferior frontal gyrus, middle frontal gyrus, superior parietal lobule and supra-marginal gyrus. The comparison subjects and FXS participants did not differ significantly on either the 1-back or 2-back task, however a significant group-by-task interaction was reported as being driven by the significant difference in the comparison group between the 1-back and 2-back tasks; a difference not seen in the FXS group. The increase in activation associated with the increase on working-memory load in the TD group suggests the recruitment of more neural resource in light of greater cognitive challenge. That this finding was not seen in the FXS groups suggests that perhaps the 2-back task was perhaps closer to threshold maximum performance; corroborated with the more significant drop-off in task performance in the FXS group compared to the TD group. The study further reported correlation between FMR1 gene activation ratio, and brain activation in right inferior frontal gyrus, left middle frontal gyrus, and right middle frontal gyrus; and FMRP expression and brain activation in right inferior frontal gyrus, left middle frontal gyrus, right middle frontal gyrus, left supramarginal gyrus, and right supramarginal gyrus.

Greicius et al (2004) examined brain activation in a group of 12 females with FXS during a visual-encoding task compared to 16 age-matched, typically-

developing control subjects. The main finding was of significantly reduced activation in the FXS group in the left hippocampus and left basal forebrain during successful encoding of visual scenes. However, the FXS group also showed increased activity compared to controls in three clusters including superior parietal region bilaterally and the right precentral gyrus; although further exploration revealed the precentral gyrus result was driven by greater deactivation compared to baseline in the TD group.

Studies of attention/response inhibition

Menon et al (2004) reported the results of a Go/NoGo task used to probe the neural underpinnings of response inhibition in a group of females with FXS, compared to an age- and gender-matched typically-developing control group. In the task, participants in the 'Go' condition had to press a trigger upon each visually-presented letter, and in the 'Go/NoGo' condition had to respond with a trigger press to all letters except the letter 'X'. Their main finding was of different patterns of activation in response to the task, with significantly reduced activation in the FXS group in the left and right supplementary motor area, right anterior cingulate and midcingulate cortex, right putamen, left and right thalamus, right middle and inferior temporal gyri. There were no regions of increased activation in the FXS compared to the TD control group. They further reported a number of clusters of activation that positively correlated with FMRP levels, including the left cerebellum, right ventrolateral prefrontal cortex and the left and right striatum in the FXS group.

In a similar study to that of Menon (2004), Hoeft et al (2007) used the same Go-NoGo task to examine response inhibition in a group of males with FXS. Notably, the 10 males in the FXS group were compared with 10 age- and gender-matched typically-developing controls as well as 10 age-, gender- and IQ-matched controls. Further, this study examined a FXS group with the lowest mean IQ (59.2); and thus likely better represents the full expression of FXS. As with the Menon (2004) study, they report a number of areas of significantly reduced activation in the FXS group, principally the right ventrolateral prefrontal cortex (VLPFC) and right caudate head compared to both control groups. They also reported increased left VLPFC activation in the FXS group compared to both control groups. They used a number of correlation analyses to further probe the relationships between task performance and regional activation. In controls right VLPFC activation correlated with performance, whereas in the FXS group it was left VLPFC activation that correlated with performance.

In their study of attention-switching Cornish et al (2004) used a Go-Wait task to explore the neural correlates of attention in three females with FXS, noted to be in the 'mild-normal' range of intellectual (dis-)ability Twelve typically-developing females were used as a comparison group. The results of the study mainly focus on within-group analyses although regions of significantly increased activation in the cingulate cortex and left and right prefrontal areas were seen in controls compared to the FXS group with no corresponding regions of greater activation in the FXS group.

Studies of equivalence processing

Klabunde (2015) used a task looking at the neural correlates when undertaking a task of identifying equivalence between pie charts, fractions and decimals. Although on face value a mathematical task, the authors were attempting to probe a more general construct of transitivity/equivalence. The subjects included 8 participants (6 female, 2 male) with FXS and a comparison group of 10 age-and IQ-matched controls. Participants were initially trained on the equivalence of fractions to pie-charts and on pie-charts to decimals, with only participants able to meet >80% accuracy taken forward to the imaging study. In the in-scanner task, participants were asked to identify which of three possible stimuli was equivalent to a presented stimulus. In addition to the comparisons they had trained on, they were also asked to identify equivalence in the novel comparison of fractions and decimals. In this last contrast, the FXS group showed clusters of significantly greater activation in the right middle temporal gyrus, left superior frontal gyrus, left precuneus, and left paracentral lobule. The authors note that these regions are more typically associated with mathematical skills than equivalence-formation and suggest that the FXS participants may be using alternate strategies for the task.

Studies of arithmetic processing

Arithmetic processing is an area of cognition that is reported to be a domain of relative cognitive difficulty in both FXS as a whole, but also specifically in females with FXS (Murphy & Mazzocco, 2008). In their study, Rivera (2002)

sought to investigate the brain circuitry used during this procedure, and whether there was any relationship with FMR1 expression. Their FXS group comprised of 16 females, who they compared with 16 female age-matched controls. Their in-scanner task involved participants assessing two- and three-operand arithmetic equations and making a judgement as to whether the given answer was correct. Overall, the FXS group showed similar performance on the two-operand equations but significantly poorer performance on the three-operand equations. As regards the activations seen during the task, when considering the extra activation attributable to increasing complexity of task, the control group showed significantly more activation in left superior frontal gyrus, bilateral motor cortex, bilateral superior parietal lobe, right intraparietal sulcus, left angular gyrus, left fusiform gyrus, left middle occipital gyrus, left cerebellum and right middle frontal gyrus. In addition, they identified a number of areas in the FXS group where the FMRP level correlated with activation. Regions correlating with 2-operand activation included: right orbitofrontal and middle frontal gyrus, bilateral cingulate cortex, left pre-supplementary motor area and left cerebellum; whilst FMRP level correlated with 3-operand activation in bilateral middle frontal gyrus, left inferior frontal gyrus, right postcentral gyrus, right superior frontal gyrus, left motor/premotor cortex, and left caudate. At a lower (voxel-wise) significance level in a priori regions, FMRP also correlated with a cluster of activation in the left supramarginal gyrus / angular gyrus. As with previous studies, this finding of increased activation in line with increasing FMRP levels, suggests that activation in these regions is a feature of more

typical brain development, and ties in with the between-group findings of reduced activation in the FXS group.

Studies of cognitive Interference

Tamm et al (2002) examined the neural correlates of a cognitive interference task, comparing 14 females with FXS and 14 age-matched typically-developing controls. The in-scanner task was a counting Stroop task, which involved participants responding with a button-press corresponding to the number of letters in the word presented. In the two conditions, either the word 'fish' was presented (neutral condition) or one of the words, "one", "two", "three" or "four" was presented (interference condition). In their analyses, the authors attempted to identify activations associated with the interference component of the task. Controls showed significantly more activation than females with FXS in the right orbitofrontal gyrus, left insular cortex / orbitofrontal gyrus / frontal operculum, and the left superior temporal gyrus / superior temporal sulcus. In contrast, the FXS group did not show any clusters of significantly greater activation than controls. As with other studies, the two groups differed significantly on IQ, which in this study was used as a covariate of no interest in the analyses.

Studies of functional connectivity

As previously described in the review of autism imaging, functional connectivity has also been used in the fragile X imaging field. In Hall et al's (2013) functional connectivity study in FXS they used both fractional amplitude of low-frequency

fluctuations (fALFF) analysis and group-independent component analysis with dual regression to identify large-scale resting-state networks. Compared to 16 age-, IQ- and behavioural/cognitive symptom-matched controls, in their group of 17 individuals (9 female, 8 male)) with FXS they reported decreased functional connectivity in salience, precuneus, left executive control, language and visuospatial networks. The FXS group had one cluster in the left thalamus of the primary visual network with greater connectivity compared to controls. In their fALFF analyses they found decreased fALFF in bilateral insular, precuneus and anterior cingulate cortices in their FXS participants compared to controls. In correlation analysis, they found that IQ in the FXS group correlated with fALFF in the left insular cortex. In a parallel voxel-based morphometry analysis, the authors also reported decreased grey matter density in the left insular cortex; suggesting from this convergence of findings that the left insular cortex may be an area that might be an 'imaging biomarker for FXS'.

Discussion

Differential activation

There are a few key messages that come out of the previous functional imaging studies in FXS. Across all the contrasts considered, there are more clusters identified of lower activation in FXS than there are clusters of higher activation. Considering the face, gaze and emotion studies in particular, there were 22 clusters identified of significantly greater activation in controls compared to the FXS groups (Dalton et al., 2008; Garrett et al., 2004; Hagan et al., 2008; Holsen et al., 2008; Kim et al., 2014; C. Watson et al., 2008), with only 10 clusters of greater activation identified in FXS groups compared to controls (Dalton et al., 2008; Garrett et al., 2004; Hagan et al., 2008; C. Watson et al., 2008). As well as these quantitative differences there were also qualitative differences, with a number of the studies identifying differing patterns of activation. For example, in Garrett et al's (2004) study whilst identifying clusters of significantly greater activation in both controls and FXS participants, the control participants showed a pattern more consistent with the expected pattern of activation, whereas the FXS group showed an atypical pattern.

Correlations with FMRP/FMR1 expression

In addition to whole-brain and region-of –interest analyses, a number of the studies reviewed also considered correlations between activations and FMRP levels or FMR1 expression. These analyses give potential further insight into

regions where the impact of FXS may be greatest; as well as helping to unpick some of the issues inherent when using, either exclusively or predominantly, individuals who are less cognitively affected. In most cases, the correlations identified clusters, which had typically been identified as being hypoactive in FXS at the group level, in which increased activity was associated with higher (i.e. more typical) levels of FMRP (Bruno et al., 2014; Hagan et al., 2008; Kwon et al., 2001; Menon et al., 2004; Rivera et al., 2002). Although Hall et al (2009) found no regions that correlated with activation and Hoeft et al (2007) only found that FMRP levels mediated the relationship between activation in the right caudate head and the left ventrolateral prefrontal cortex. Where FMR1 mRNA was measured, similar positive correlations between higher (i.e. more typical) activation ratios and clusters of activation were found (Kim et al., 2014; Kwon et al., 2001).

Complexity

In studies that explored brain activation during tasks of increasing complexity (Klabunde et al., 2015; Kwon et al., 2001), it is interesting to note that on the simpler level of tasks there was typically no differences seen in activations between FXS and control groups; however, it was on increased complexity of task that the differences emerged. In the study of working memory, the differential activation on increasing n-back task complexity was associated with a significant difference in performance on the task (Kwon et al., 2001), whereas in the task probing emergent equivalence relations, there was no group

difference in task performance at either level, but nonetheless a significant difference in activations at the higher level of complexity (Klabunde et al., 2015).

These suggest a number of possible mechanisms; in the case of a significant drop-off in performance on the working memory task, the differential activation may reflect a combination of the greater cognitive effort required to complete the task and the FXS group operating at a 'ceiling' level of complexity. It may also reflect a behavioural difference – that with increasing complexity, if a participant feels the task is now beyond their ability, they may disengage from further engagement in the task, leading to differential activation. The findings from Klabunde et al (2015) potentially also fit with this, in that although there was no difference in performance, it was the FXS group that showed excess activations compared to controls; suggesting that the individuals in the FXS group needed to recruit extra cerebral resources to keep up performance-wise.

Conclusions

The first issue that arises is the degree to which individuals who are both invited to participate, but also who ultimately make it into the analyses, are representative of FXS more globally. The over-representation of females with FXS, as well as individuals of higher cognitive ability is clearly an issue for the field of research. The use of correlation analyses with both IQ and FMRP/FMR1 expression go some way to try and unpick this, however, as with functional imaging studies in autism, further endeavour is required to try and address the

selection bias in the field. On a qualitative level, it was evident that in a number of papers there were different activations seen in both comparisons considered (e.g. TD>FXS and FXS>TD), however, more commonly only the TD>FXS results were referenced in the abstract. Whilst acknowledging that abstracts are limited in what they can report, it introduces the potential for bias in synthesizing results, and may present a challenge to the field.

Despite some suggestions in the research of possibly identifying an, “imaging biomarker” of FXS, it is likely that we are probably still some distance from that. As it stands, the heterogeneity of tasks studied to date, and the relative lack of replication of previous findings, suggests that further work is required to try and hone a more coherent thesis on any core differences.

Future directions

Bringing together the findings from the two imaging reviews in this and the previous chapter, it is clear that there is generally an issue of the representativeness of the participants in many of the studies. Further, beyond the selection of the main group participants in these studies, there are issues about the choice of control groups, and whether, and to what degree, important baseline characteristics, in particular IQ, are taken into account. Thus, there is a need for studies in this field to consider how functional imaging can be undertaken in individuals who are more intellectually impaired. The next two chapters are experimental studies, which aim to take the field a little closer to answering some of these questions, and to shine a light on the issue of whether the findings to date generalise to any degree in groups of individuals who are more intellectually impaired.

Chapter 4: Functional Imaging Experimental Methods

Introduction

In considering the results of the reviews of functional imaging, the following hypotheses arose:

1) That whilst likely to be difficult, it should be feasible to undertake functional scans in individuals of more limited cognitive ability.
2) That it would be possible to elicit a BOLD response to faces over and above a baseline condition.
3) That in both SEN and FXS cohorts, for subgroups with high autistic traits, there would be relative hypoactivation to fearful faces.

With these in mind, the studies described were developed to test these hypotheses. Elements of the design and the choice of measures made will be reflected upon throughout the methods chapter. In order to avoid undue repetition, this chapter covers the experimental methods used in both functional imaging studies. Whilst in many cases the methods were the same, where there were differences these are noted.

Ethical permission for the studies

The two studies were approved by the National Research Ethics Service Scotland 'A' Research Ethics Committee in Edinburgh; this being the committee with the remit for reviewing studies which include individuals who may not be able to consent for themselves. In both of the imaging studies, there were the options for either the participant themselves to consent, or

where it was assessed that they lacked the capacity to consent, for a welfare guardian or nearest relative to consent on their behalf.

Establishing capacity to consent

One of the focuses of both the pre-visit preparatory contact, and also the early part of study visits was to establish the participants' capacity to consent. Under the Adults with Incapacity (Scotland) Act 2000 incapacity is defined as being:

"incapable of—
(a) acting; or
(b) making decisions; or
(c) communicating decisions; or
(d) understanding decisions; or
(e) retaining the memory of decisions,
… by reason of mental disorder or of inability to communicate because of physical disability."

Assessments of capacity to consent were completed by the researcher (Andrew McKechanie), who is recognised by the local Health Board under section 22 of the Mental Health (Care and Treatment) (Scotland) Act 2003 [as amended] as "having special experience in the diagnosis and treatment of mental disorder". Under section 51 of the incapacity legislation, special authority for medical research is given under the following relevant conditions:

(1) research of a similar nature cannot be carried out on an adult who is capable in relation to such a decision; and

(2) the purpose of the research is to obtain knowledge of the causes, diagnosis, treatment or care of the adult's incapacity; and

(3) (a)the research is likely to produce real and direct benefit to the adult;
(b)the adult does not indicate unwillingness to participate in the research;
(c)the research has been approved by the Ethics Committee;

(d)the research entails no foreseeable risk, or only a minimal foreseeable risk, to the adult;
(e)the research imposes no discomfort, or only minimal discomfort, on the adult; and
(f)consent has been obtained from any guardian or welfare attorney who has power to consent to the adult's participation in research or, where there is no such guardian or welfare attorney, from the adult's nearest relative.

(4) Where the research is not likely to produce real and direct benefit to the adult, it may nevertheless be carried out if it will contribute through significant improvement in the scientific understanding of the adult's incapacity to the attainment of real and direct benefit to the adult or to other persons having the same incapacity, provided the other circumstances or conditions mentioned in subsections (1) to (3) are fulfilled.

Recruitment of Participants

Recruitment of SEN participants

The participants for this arm of the study were individuals who had been previously enrolled in a brain imaging study examining the characteristics of individuals receiving special education needs (SEN) support as the result of low intellectual ability (Johnstone et al., 2007). For this parent programme of research, all educational boards in Scotland had been approached, with 18 of 19 agreeing to participate. In turn, the schools in those boards were each contacted, with 99 of 273 agreeing to participate. The teachers in each of the schools were asked to identify the children receiving special education support because of presumed low intellectual ability (with an estimated IQ of 50-80, as IQ was not routinely measured in schools or SEN services). For the original study, the exclusion criteria had been those with Down syndrome or other syndromal features, major sensory impairments, absence of speech, significant cerebral palsy, and individuals with clear severe or profound intellectual disability. It was by re-contacting participants who had participated in the final scan of this parent study that participants were recruited to the present study.

Recruitment of fragile X syndrome participants

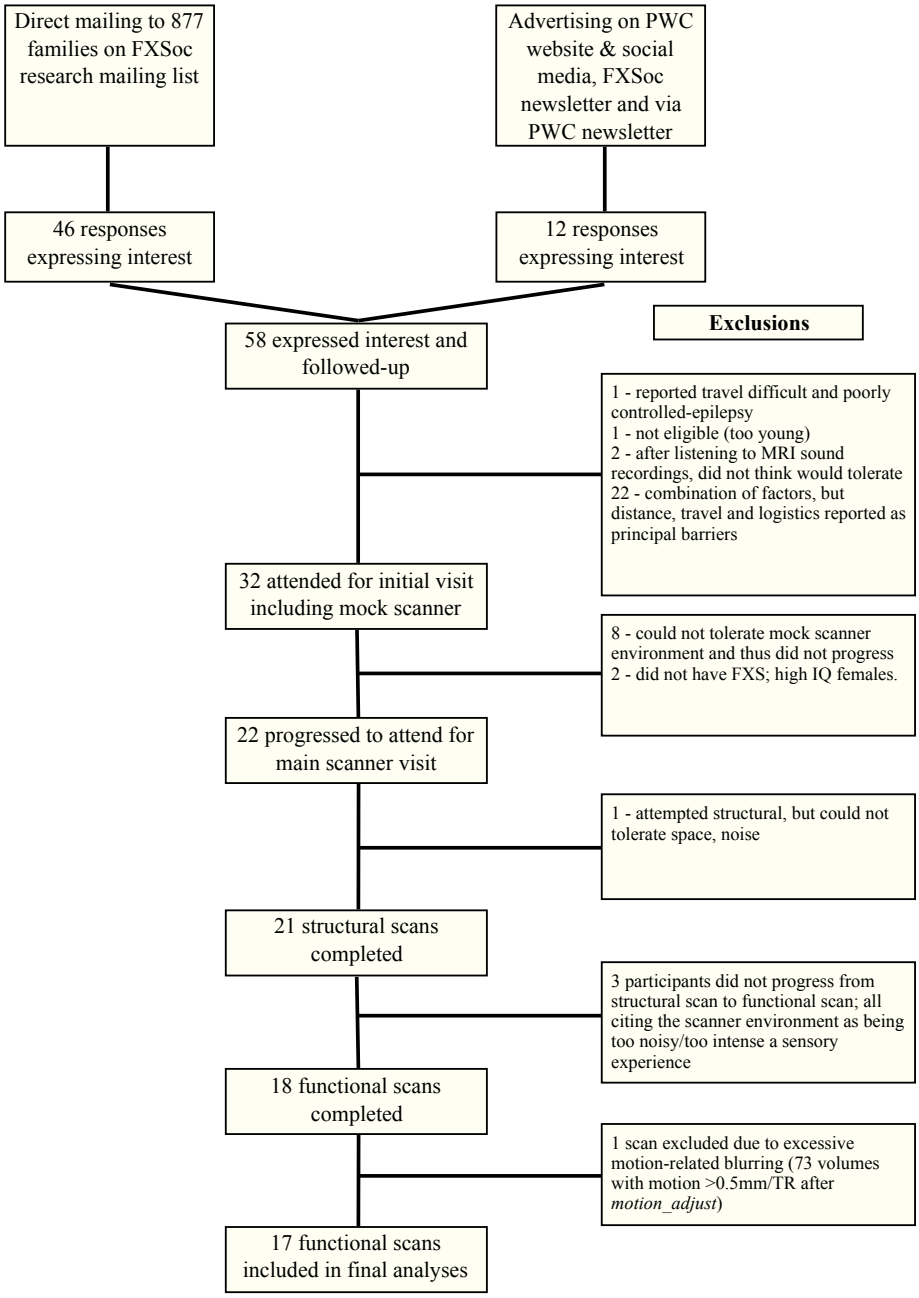
Initial recruitment was through the Fragile X Registry at The Patrick Wild Centre in Edinburgh. However, with the support of the UK-based family support charity, The Fragile X Society, the study was advertised in their quarterly print newsletter and on their website. Subsequently, mailshots to families registered with the society as being interested in research were sent out. Each mailshot included a study information leaflet, an invitation to participate letter from the researcher, a cover letter from the Fragile X Society and a response sheet for those who wished to respond by letter rather than by telephone or electronic mail.

The initial mailshot went to the northernmost 349 families registered with the society, with a subsequent mailshot going to a further 528 families living in the rest of the UK.

Figure 4 shows the numbers who came through the recruitment from first contact to completed scan. As can be seen, most who managed to enter the scanner were able to complete the sequences, with only 4 dropping out once they had stared the sequence.

Figure 4

Flow diagram showing the recruitment of FXS participants from initial approach to inclusion in final analysis.



Note. FXSoc, Fragile X Society; MRI, Magnetic Resonance Imaging; PWC, Patrick Wild Centre.

Practical arrangements of study participation

There was significant variability in the level of support required to allow participants to participate in the study. For those in the SEN study, the majority of participants (15/18) attended with a supporter of some form (most typically a parent). Of the other three participants, two attended with a peer for companionship on the study visit, and one attended on their own. For those with fragile X syndrome, all included in the final analyses attended with a supporter, most commonly a parent.

In most cases, participants and their families arranged their own transport and, where necessary, accommodation; with expenses being refunded by the study budget. However, in a number of cases travel and accommodation were organised directly by the researcher, facilitating access to the study especially for those perhaps less accustomed to travel. Taxi transport was arranged by the researcher to ease attendance for those in hotel accommodation, and, for those arriving by car, visual information on how to find the research sites was provided.

MRI methodology

Scanning procedures

All participants were scanned at the Clinical Research Imaging Centre (CRIC) of the University of Edinburgh at the Royal Infirmary, Edinburgh campus. After the lead radiographer had reviewed their subject screening questionnaires, participants were taken through to the controlled area of the centre, usually, but not always, accompanied by a parent or relative. There, they had their height and weight measured by the radiography staff for calibration of the scanner. In most cases, participants had been able to find clothes of their own to wear in the scanner which were metal-free, however, for the others jogging bottoms and t-shirts or gowns were provided to change into.

All scans were completed on a Siemens MAGNETOM Verio 3T scanner operated by the radiography staff at the CRIC, whilst the researcher operated the computer that presented the stimuli for the functional scans.
For the structural imaging, using a 5min 3s MPRAGE sequence, a T1 structural image was obtained made up of 160 coronal slices of 1mm slice thickness and 1mm x 1m x 1mm voxels. A repetition time (TR) of 2.3s, an echo time (TE) of 2.98ms, flip angle of 9° and field of view (FOV) of 256mm were used as advised by the medical physicist in CRIC. For the functional scan, which was only 4min 13s long, 159 volumes were acquired; each containing 26, interleaved, 5mm slices of voxels 3.4mm x 3.4mm x 5mm. In this case a TR of 1.56s, a TE of

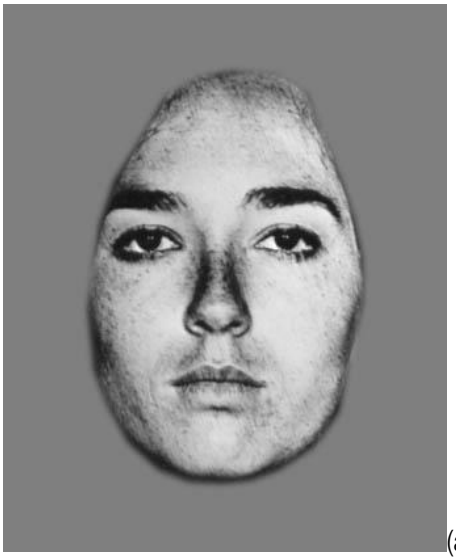
26ms, flip angle of 66° and FOV of 220mm were used, again as advised by medical physics colleagues.

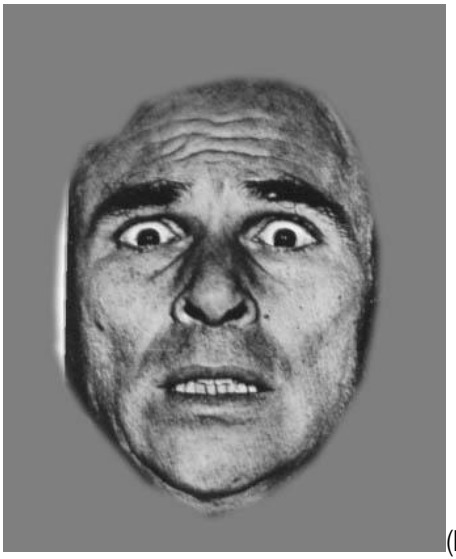
Functional MRI task

The task used in this series was a block-design task with two main conditions including a series of neutral faces, and a series of fearful faces, the faces being taken from the Pictures of Facial Affect series (Ekman & Friesen, 1976). A visual fixation cross was presented at the beginning and end of the sequence, as well as between the conditions of interest. This being considered as the baseline condition for contrasts against baseline. The complete sequence presented 6 blocks, each of six faces alternating between blocks of fearful or neutral faces. Within each block, each face was shown for 3.5s with an inter-stimulus interval of 0.5s. In between each block was an interval of 12.5s during which a fixation cross was shown. There were two variations of the sequence, with one starting with a block of neutral faces and the other starting with a block of fearful faces. Which sequence was used was balanced across the participants. As had been rehearsed in the mock scanners, participants were asked to depress a trigger button each time they saw an image. This was principally used as an in-scan method for ensuring participants were attending to the task. All participants successfully depressed the trigger for >80% of faces shown. Figure 5 shows examples of the neutral and fearful faces used.

Figure 5

Examples of the neutral (a) and fearful (b) faces from the Pictures of Facial Affect series


(a)


(b)

Selection of paradigm

Whilst in the prior reviews a wide variety of paradigms were used to test a number of different questions; emotion processing, and in particular neural response to faces, was one of the most frequently tested responses. Whilst the studies described herein sought to examine those of lower cognitive ability (who have largely been excluded from prior studies), and thus were seeking to do something somewhat novel; the decision was made to keep many of the other elements of study design more standard, so as to reduce other sources of variability. The Pictures of Facial Affect series (Ekman & Friesen, 1976), whilst having its limitations, has the benefit of having been widely used in prior studies

(including many studies of autism). Further, it has previously been used in other studies of autism within the department the research was conducted in, opening up the possibility of combining data at a later stage in order to be able to answer further questions about the nature of the underlying biology of autism.

The block-design nature of the paradigm was arrived at largely for simplicity of administration to the participants. Using a fixation cross as the baseline condition is the method that has been typically used in other studies within the department and its use for this experiment was in part to allow future combination of data. It was also used to, hopefully, evoke a robust BOLD response on the faces/neutral/fearful>baseline contrasts which could be measured and analysed. The inclusion of the more subtle fearful>neutral contrast was also included to allow for a more in-depth analysis of differential response. It would have been possible to have used an alternative baseline image (e.g. an equal-luminance geometric shape or a pixelated face), however, it was felt that there was the potential for even this to be too subtle a contrast and that it may not have elicited a sufficient neural response for analysis. However, in light of the results presented further on in the thesis, it could be considered for future studies.

Scanning subject screening questionnaire

As with all MRI studies, a scanning subject screening questionnaire was required to be completed for each participant and, where accompanied into the scanner by a parent/guardian, one was completed for them as well. This checklist was then checked and signed off by the supervising radiographer and any possible issues explored further. As the MRI exclusion criteria had been explored with all participants ahead of their scans, nobody needed to be excluded as a result of their questionnaire answers.

Preparation for Fragile X research scans

Challenges of having a scan

Whereas the SEN participants had already had 3 previous research structural MRI scans and thus were familiar with the process, the scanning process was novel to all the fragile X participants. Having an MRI is a potentially highly anxiety-provoking procedure for any participant, with non-completion due to claustrophobia being reported in 1-15% (mean 2.3%) undergoing diagnostic imaging in the general population (Dewey, Schink, & Dewey, 2007). However, for participants with an intellectual impairment or disability there are further barriers. Indeed, for those with fragile X it appears as if many of the common characteristics of the syndrome align perfectly with particular challenges of scanning. As seen in chapter 3 it would appear that many of the most intellectually affected are not included in much of the research, or given the

challenges, other modalities are used; e.g., as described by Ballinger et al (2014). The four principal challenges that were identified as being barriers to successful participation were: anxiety in new environments, auditory hypersensitivity, hyperactivity and difficulties understanding and consenting to the study.

Anxiety in new environments

Anxiety is a common feature in individuals with fragile x syndrome (Cordeiro, Abucayan, Hagerman, Tassone, & Hessl, 2015), with the manifestations of anxiety being commonly provoked by environmental stressors and less common in familiar settings (Tranfaglia, 2012). Thus, participating in a study of any form in a new environment and with unfamiliar staff poses very significant potential difficulties to be addressed.

Auditory hypersensitivity

Individuals with fragile X are known to have increased sensitivity to noise, with electroencephalogram (EEG) and magnetoencephalogram (MEG) evidence of greater amplitude, increased latency and reduced habituation in auditory event-related potential studies (Rojas et al., 2001; Schneider et al., 2013). In the context of this known hypersensitivity to sound, completion of an MRI is a particular challenge. The scanner used had a peak sound pressure level (SPL) of 115 dB(A), which for context is a similar volume to a high-power (1600W)

hammer drill (The National Institute for Occupationsal Safety and Health, 2006) or twenty times the SPL of a domestic vacuum cleaner.

Hyperactivity

Hyperactivity is a common feature of fragile X syndrome (Sullivan et al., 2006) which in the context of an MRI potentially poses significant issues. Image quality is inversely proportional to the degree of movement during the scan and whilst some motion artefact can be managed in the pre-processing of the images, it was important to consider strategies to minimise movement during image acquisition.

Difficulties understanding and consenting

This poses an issue at two different levels: firstly the ability to understand what is going to happen to the participants, to allow them to express choices and exert autonomy; but also at the level of trying to pragmatically facilitate what is going to happen to them, and to prepare for the procedures. This ties back to the earlier issue of anxiety in new environments and work done to prepare the participants as best as possible would be work well done in trying to reduce any anxiety that could be reasonably expected.

Procedures to facilitate participation

In order to try and reduce the impact of these challenges associated with the scanning protocol, a number of procedures were incorporated.

Adapted information materials

Prior to the visit participants and their families were provided with visual information sheets which showed the buildings they would be coming to, an image of the scanner and a picture of the researcher (Andrew McKechanie) they would meet on the day. Audio recordings of the scanner sequences, were also sent to participants so that they could listen to the sounds in their own home ahead of their scan, to reduce the novelty of the sounds.

Rehearsal

On the day of the scan, participants were able to rehearse on one or both of the mock scanners. The first mock scanner was housed in the Department of Psychiatry and had been built for the previous SEN cohort study. Made of medium density fibreboard, it had a 62cm bore (smaller than the 70cm of the real scanner), with a full-sized head-coil and a sliding bed. It was also fitted with a TV monitor capable of showing images visible to the participant to simulate the experience of the real scanner. Earplugs and headphones were used to further simulate the experience and the aforementioned sequence sounds

could be played through the headphones to complete the sensory experience. Plate 1 shows the mock scanner in The Patrick Wild Centre.

The second mock scanner was a full-sized replica of the real scanner, with the exception that it did not have a main magnet, and thus could be used without pressure of time. On this mock scanner participants were able to experience the motorised bed and the scale of the real scanner, along with similar lighting to the real scanner. Plate 2 shows this mock scanner at the Clinical Research Imaging Centre.

When using the mock scanners, the researcher first demonstrated their use on himself before allowing the participant to try it. In most cases this was done in a staged procedure. First, the participant would lie on the bed outside of the bore and slowly acclimatise to the experience. This was followed by slowly sliding the bed into the mouth of the bore (without the head coil on), and then proceeding deeper into the bore (again without the head coil). After a period of acclimatisation, the participant was then slid out, and now tried on the head coil outside of the bore. Once the participant was happy inside the head coil outside of the bore, they were slid slowly into the bore, both observing for signs of, and enquiring about, any anxiety. Once they had acclimatised to this they were removed from the bore before going back in first with the headphones on with no sound playing, and then with the headphones on whilst listening to the recorded sounds of the scanner. Finally, the participants were able to view the

research paradigm (with different facial stimuli to the real task) on a screen and use replica triggers as in the real scanner. Plate 3 shows one of the fragile X group participants on the mock scanner at the Clinical research Imaging Centre.

Plate 1

Mock scanner housed within The Patrick Wild Centre, where participants could first encounter and rehearse scanning procedure.

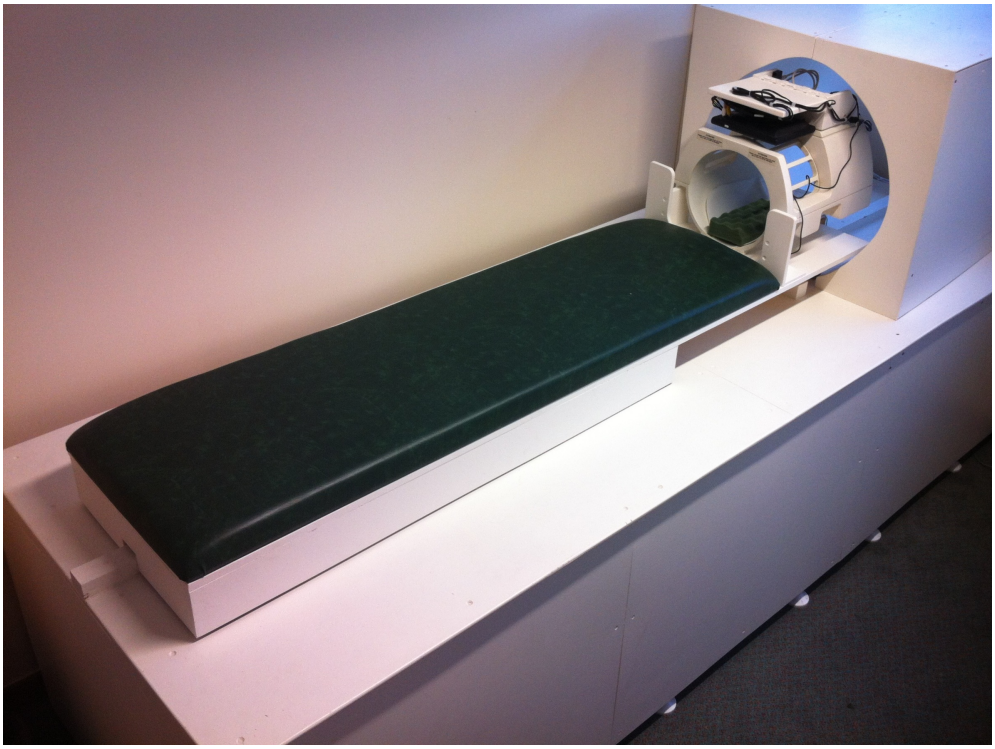


Plate 2

Mock Siemens Magnetom Verio (no coils), housed at the Clinical Research Imaging Centre, Edinburgh.

Plate 3

Participant rehearsing on the mock scanner at the Clinical Research Imaging Centre, Edinburgh, supervised by Andrew McKechanie.

Note. Image reproduced with permission of the participant and legal representative.

Considerations during scanning

To help reduce any distress that may be associated with auditory sensitivity, participants were given a choice of earplugs and headphones to be used during the scan. Further, in choosing the sequence, it was decided to keep the sequence as short as possible. Thus, the full scanning sequence was less than 10 minutes duration, comprising of two ~5 minute sequences (one functional and one structural). Where participants found it helpful, they were able to be accompanied by a parent/guardian, who was able to remain either in the scanning room, or by their side during the scans. Whilst in almost all cases the two sequences were run one after the other (with the researcher speaking to the participant in between sequences to check they were happy to continue), on 4 occasions, the participant requested a brief rest between the sequences. In these cases, all that was required to accommodate this was a second localizer sequence to be run prior to the second scan. The radiographers were expert in working with the participants to allow them to feel comfortable and used a variety of techniques to facilitate their scans. The most important consideration was that for each of the 10 minutes of scanning slots, a 60-minute slot was actually booked (and budgeted for), so that there would be no time pressure on any of the participants, their supporters or the radiographers to complete a scan.

Whilst many of these adaptations were principally designed to help better accommodate the individuals with fragile X syndrome, it was clear that they

have wider applicability and that there may be elements which, if incorporated into mainstream scanning protocols, may reduce scan failure rates. Some of these are already included in paediatric settings, but less so in mainstream imaging practice or research studies.

Test materials / procedures

Measures of autism

In this study the main measure of autistic traits was the second version of the Autism Diagnostic Observation Schedule (ADOS). This is a direct observational measure based on an interview with each participant. In the case of two participants in the SEN cohort, where an ADOS was not completed, their scores on the SCQ which had been completed by their primary caregiver in the previous study was used to categorise them into the ASD or non-ASD subgrouping. Whilst the ADOS allows for, and indeed provides scoring suggestions for, the scores to be converted into diagnostic categories, it also lends itself to the exploration of autistic traits as a continuous variable, both of which are covered in the experimental chapters.

The Autism Diagnostic Observation Schedule (ADOS) – 2nd version

The ADOS-2 (subsequently referred to as simply the ADOS) is a semi-structured interview that uses a set of prescribed "presses" to elicit, demonstrate or create the space in which autistic features may be assessed either by the presence or absence of features which are useful in helping to establish an autism diagnosis (Lord et al., 2012). This format allows for the assessment of autistic and associated features including 31 items across 5 domains. The 5 domains include the 3 main domains considered in autism diagnosis (social, communication, and stereotyped behaviours and restricted

interests) plus the related domains of creativity and “associated features”. Interviews were completed with participants to directly assess autistic traits and to categorise the participants into an autism or non-autism group. For each participant, a combined social and communication total of ≥10 was used as a cut-off to divide the group into ‘FXS’ and ‘FXS + autism’ groups for the between-group analyses. For the purposes of the regressions against measures of autistic symptomatology the Calibrated Severity Score (CSS) was calculated for each participant using the published algorithms. In the case of the participants who were scored on the ADOS module 4, the CSS algorithm subsequently published by Hus & Lord (2014) was used.

The Social Communication Questionnaire (SCQ)

For the participants in the SEN imaging study, the SCQ had been completed by their caregiver in the parent study. Thus, for the two participants who did not complete the ADOS, their SCQ scores were used to categorise which subgroup to allocate them to, as detailed in chapter 5. The SCQ is a 40-item caregiver-completed rating scale that was designed by the authors as a questionnaire-based tool, based on the Autism Diagnostic Interview – Revised (ADI-R) as a proposed screening tool for autism (Rutter, Bailey, & Lord, 2003). A score above a cut-off of 15 is commonly seen as suggesting the individual is likely to have ASD, with differing cut-offs being used depending on the characteristic that is valued more highly (sensitivity vs. specificity). It comes in Lifetime and Current versions, which can be used to either inform ASD

diagnostic status or current difficulties. The individual item scores can also be totalled to give Social Relating, Communication and Range of Interests subscale scores, although these are less commonly used.

Selection of autism assessment tools

Whilst in clinical practice, the gold standard for assessing autism would be a multi-disciplinary clinical assessment, including a full developmental history and often taking a number of consultations, and in some cases utilising standardised questionnaires or assessments; it was not feasible to include this within the protocol of this study. Thus, a pragmatic decision was made to use the semi-structured assessment tool, the ADOS-2, for assessment of autistic traits. It has been widely used in previous studies of both autism and fragile X syndrome although, as discussed later, there are issues arising from its use in adults with intellectual disability, where the reliability data is sparser. Nonetheless, as an acceptable, structured, clinical assessment it met the criteria required for these studies.

In the two cases where individuals were not able to undertake the ADOS, it was fortunate that having their SCQ scores from the parent study it was possible to allocate them to their respective groups. Whilst the SCQ was not a core part of the data collected on participants, it was felt that it was reasonable to use it for

this purpose, having good reliability and validity data, and having been used in 5 of the prior studies reviewed.

Measures of cognitive ability

WAIS/ WISC

Participants in the SEN imaging study had all previously completed either of the Wechsler Intelligence Scale for Children (WISC) (Wechsler, 1992) or the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1999) as appropriate to the individual's age at the time to assess their IQ. We decided not to re-test these participants for the present study, believing that it would be unnecessarily burdensome to participants who had already undertaken previous, similar testing, and for whom we believed that any change in score would likely not make a material difference to the study.

The Kaufman Brief Intelligence Test, 2nd Edition (K-BIT-2)

For the participants in the FXS imaging study, the K-BIT-2 was used to assess IQ (Kaufman & Kaufman, 2004). This was chosen largely because of relative speed of completion whilst being considered a reasonably good measure for those with an intellectual disability (Pitts & Mervis, 2016). Upon completion, it can be scored so as to give verbal (crystallised) and non-verbal / performance (fluid) IQ scores, as well as a composite score.

Imaging analysis

Spatial preprocessing of fMRI data

Images were analysed using the Statistical Parametric Mapping (SPM) program (version 12, Functional Imaging Laboratory, Wellcome Trust Centre for Human Neuroimaging, University College London; fil.ion.ucl.ac.uk/spm/) running within Matlab (R2011b (version 7.13.0.564), MathWorks, Natick, MA, USA). Data were initially reconstructed using the DICOM Import function within SPM to convert the scanner-produced DICOM files into native NifTi files for further processing within SPM.

Prior to pre-processing, the first 7 volumes of the functional scans were discarded to reduce the impact of T1 equilibrium effects. Images were initially realigned to the mean EPI image using the Realign (realign and unwarp) module of SPM. The T1 structural image was coregistered to the mean EPI image. The T1 structural image was segmented before both structural and functional images were normalised using the normalisation parameters arising from the T1 image segmentation. This normalisation ensures that both structural and functional images are rendered in the same space allowing for inferences to be made about the anatomical location of any functional imaging results. The final step in the pre-processing of the data was to spatially smooth the functional images using a Gaussian smoothing kernel of 8mm full width at half maximum (FWHM) in each of the x, y, and z axes.

Managing scans affected by movement

SEN group

In the SEN group, the realignment parameters giving the estimates of translations and rotations of the participant's head during the functional scan were examined with the intent of excluding scans showing more than 1mm or 1degree of movement. However, none of the scans exceeded these parameters and thus no scans had to be excluded.

FXS group

One of the major impediments to imaging studies in individuals affected by significant developmental disorders is how to manage and account for movement. As noted earlier, the best way to manage these difficulties is to try and reduce the movement in the scan itself, by means of preparation, rehearsal and comfortable padding of the head during the scan. Nonetheless, there still remains the necessity to find ways to best process available imaging data, which may be significantly affected by movement artefacts. Thus for the FXS scans, a significant portion of which were affected by movement, extra pre-processing was undertaken to try and provide the cleanest data for analysis. The ArtRepair toolbox version 5b3 (P Mazaika, 2015) for SPM was used to first examine and then repair the volumes, using the motion_adjust and artifact_repair modules of the single subject pipeline described by Mazaika (2009). As above, the images were realigned, before being smoothed with a

4mm FWHM Gaussian smoothing kernel. The motion_adjust module of ArtRepair was used as an alternative to adding the movement parameters in the design matrix, as the motion regressors have been described as not being sufficiently accurate to account for the relatively larger movements of clinical subjects (P Mazaika, 2009). The images were subsequently analysed and repaired using the artifact_repair module in ArtRepair. In this step, the volumes were examined for fast head movements, and volumes with movement of >0.5mm/TR were interpolated with the nearest usable volumes. 10/17 scans had to be repaired in this way. Where a scan had >20% of volumes with >0.5mm/TR movement, the scan was excluded. Only 1 scan had to be excluded for this reason. Following this step, images were then processed using the same pipeline as with the SEN scans. The only exception being that a final 7mm kernel was used in the final smoothing step; the combination of a 4mm and a 7mm smoothing being approximately equivalent to the commonly-used single 8mm kernel.

For the comparisons between the FXS and SEN groups, the SEN imaging data was re-pre-processed using the same pipeline as the FXS data so as to be comparable; although in this group, only a single volume in a single scan had to be repaired using the artifact_repair module. Figures 6 and 7 show the pre-processing pipelines for the SEN and FXS analyses respectively. Figure 8 shows the impact of the motion correction steps on the same time series of scans in one of the participants with fragile X syndrome.

Figure 6

Imaging processing pipeline for the SEN analyses

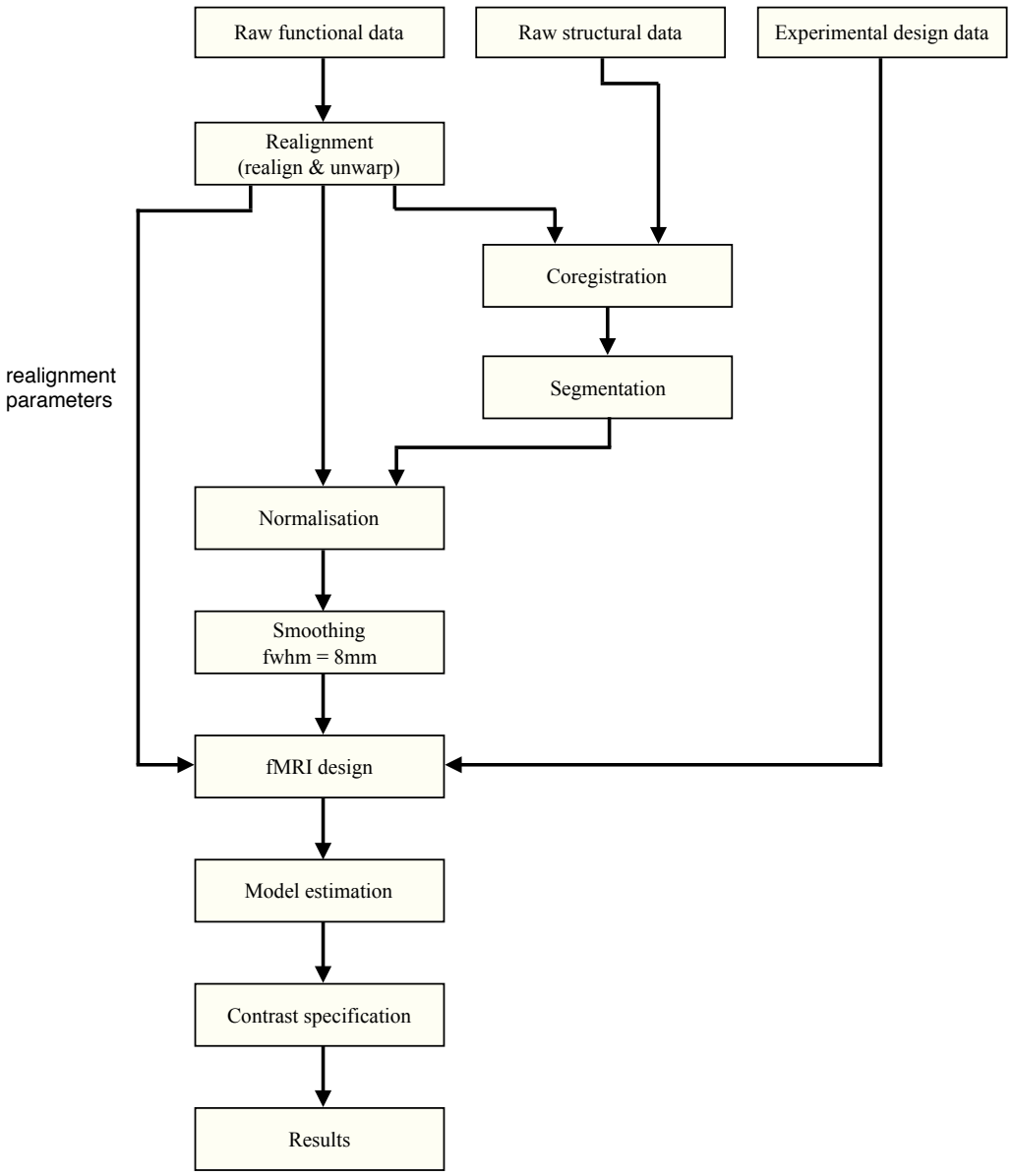
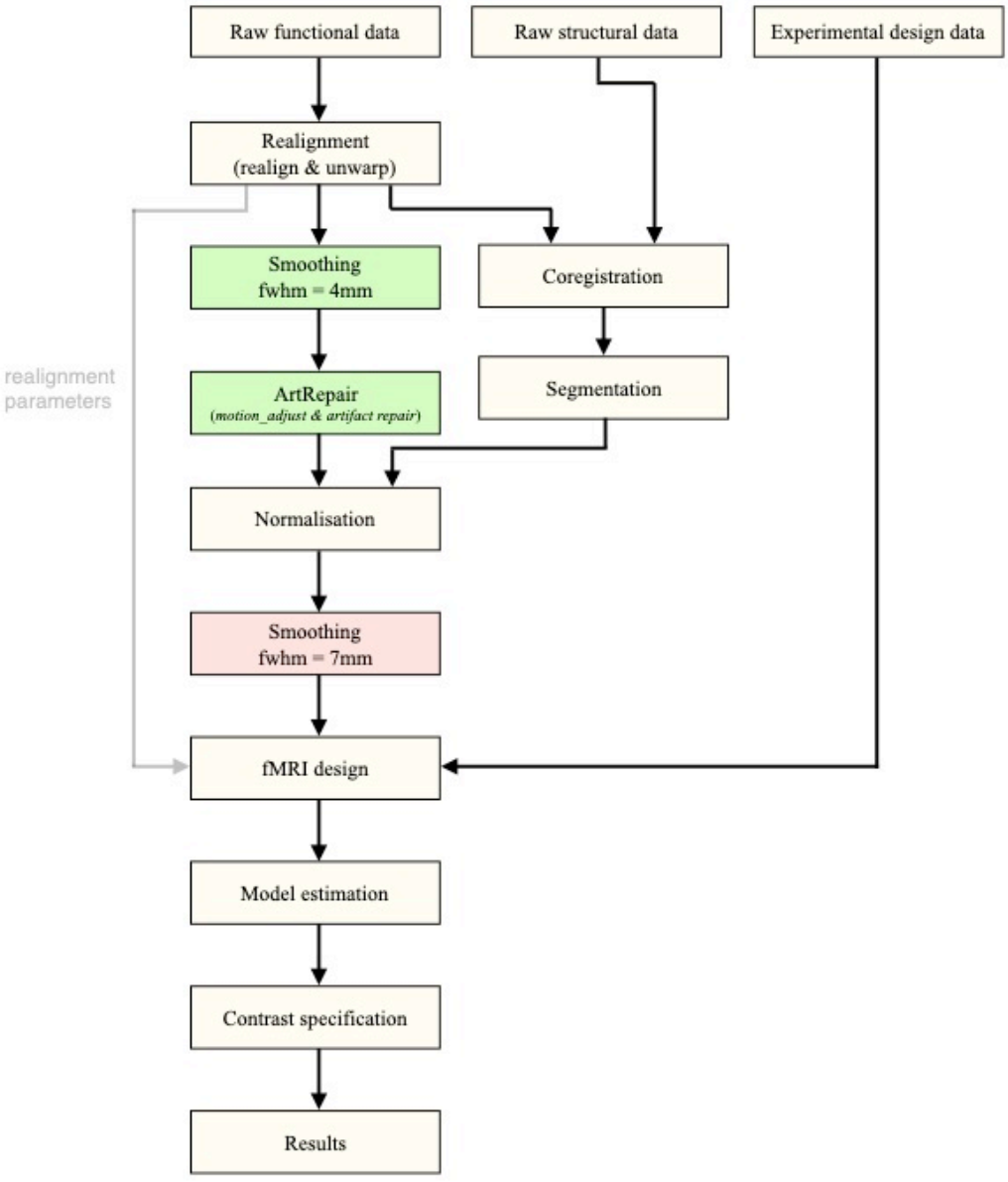


Figure 7

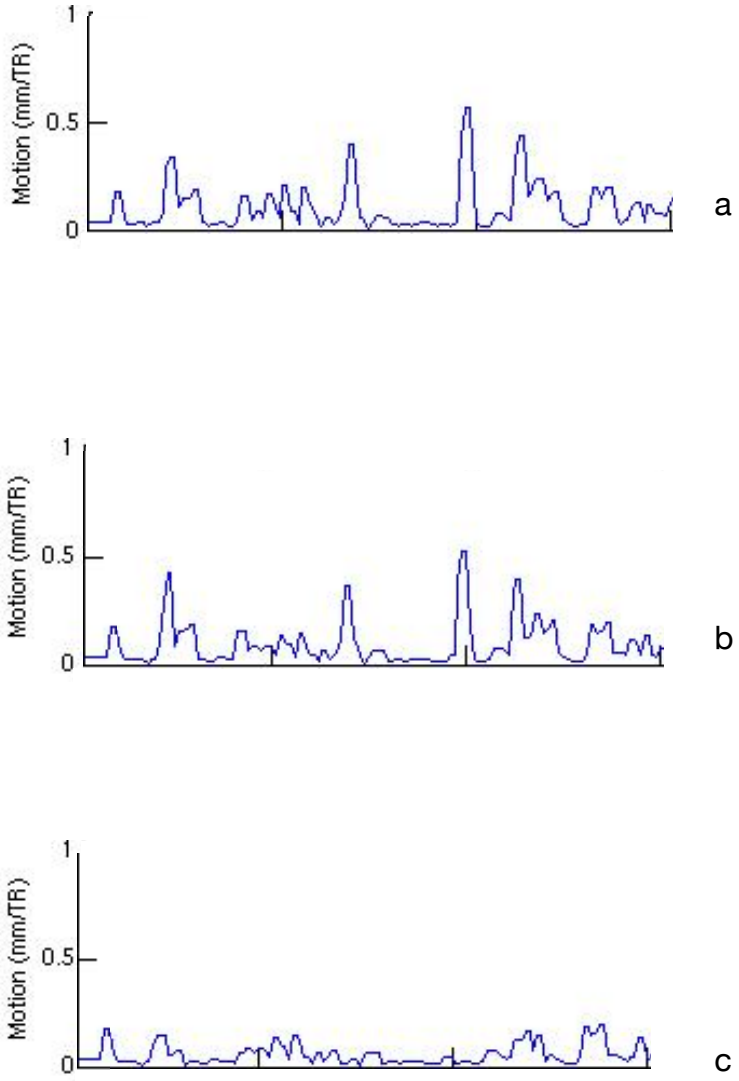
Image processing pipeline for the FXS and FXS vs SEN analyses.



Note. The green boxes denote new steps in this pipeline; the red box denotes an amended step and the transparent writing denotes the removal of the step.

Figure 8

Example of the effect of motion correction.



Note. Panels show the movement (mm/TR) in a single subject during the pre-processing pipeline.
a – smoothed images prior to *motion_adjust* module
b – images following *motion_adjust* module, prior to *artifact_repair*
c – images following *motion_adjust* and *artifact_repair* modules

Statistical analysis of fMRI data

For each contrast examined, a design matrix was created incorporating weightings for the neutral and fear conditions. For the SEN group analyses, the movement parameters generated earlier were included in the design matrix as covariates of no interest. As discussed in the preprocessing pipeline, in the FXS group these movement parameters were not included in the design matrix as the correction for motion had already accounted for head movement, as far as was possible. Contrasts were generated for each of the faces>baseline, neutral>baseline, fear>baseline, neutral>fear and fear>neutral contrasts. First-level analyses were constructed for each of these contrasts for each participant. Second-level analyses were then generated using the first level contrast images to consider differences in activation both within the groups and between the groups.

Figures were created using the montage function of SPM12. In the figures, clusters are shown overlaid on coronal sections of the single subject T1 canonical image from SPM12, with a coloured scale showing the z-score of the voxels in the cluster.

Chapter 5: A functional imaging study of autism in individuals of low cognitive ability

Introduction

This chapter presents results from the imaging analyses of the individuals with special educational needs. Each of the contrasts is considered both within the autism and non-autism groups as well as between the groups. Further analyses using a small volume correction and regression analyses are also included.

Participant details

The characteristics of participants included in the imaging analyses are shown in Table 4.

Table 4

Baseline Characteristics Of Autism And Non-Autism Groups

	Autism group	Non-autism group
N	9	9
Male : female	8:1	7:2
Age	25.2 (1.7)	23.2 (1.1)
Full-scale IQ	68 (10.9)	71 (16.0)
Verbal IQ	67 (11.1)	72 (18.2)
Performance IQ	75 (11.5)	74 (12.2)
ADOS Total*	13 (10-16)	2 (0-5)
ADOS CSS*	7 (5-9)	1 (1-2)

Note. Results show group means (s.d.) for age and IQ; and median (range) for the ADOS scores. The groups were not significantly different on gender (Chi square = 0.400, p=0.527), age (p = 0.215), full-scale IQ (p = 0.313), verbal IQ (p = 0.382) or performance IQ (p = 0.263).
*Ratings and scores based on scores for 8 individuals in each group.
ADOS, Autism Diagnostic Observation Schedule; CSS, Calibrated Severity Score.

All participants included in the analyses were medication-free and none had been diagnosed with epilepsy.

Statistical analyses

For the imaging analyses, one-sample t-tests were used for within group results, and 2-sample t-tests were used for the between group analyses. All results were thresholded at $p<0.001$ uncorrected, with results reported as family-wise error, cluster-corrected p-values. Results reported include all those with $p < 0.1$; with family-wise error-corrected $p < 0.05$ being considered significant. Results where and $0.1 > p > 0.05$ were considered as a non-significant trend as this was a small but clinically important sample and should therefore be interpreted with caution; further study or replication being required to be more confident in them. For each cluster, the significance level ($p_{FWE\text{-}corr}$), cluster extent in voxels (k_E), z-score ($Z\equiv$), and MNI co-ordinates (x, y, z) are given for the peak voxel in the cluster. Regions annotated were identified using the Automated Anatomical Labelling Atlas 3 toolbox (Rolls, Huang, Lin, Feng, & Joliot, 2019) running in SPM12. The results for the all faces > baseline contrasts, as secondary analyses, are included in Appendix 5.

Results

Within group results

Contrast: neutral faces > baseline

This contrast shows the regions of brain activity associated with viewing the neutral faces in contrast to baseline.

Non-autism group

The non-autism group showed a large region of significant activation with a peak co-ordinate in the left calcarine sulcus, and extending to bilateral calcarine sulci, bilateral lingual gyri and bilateral cuneus.

Table 5

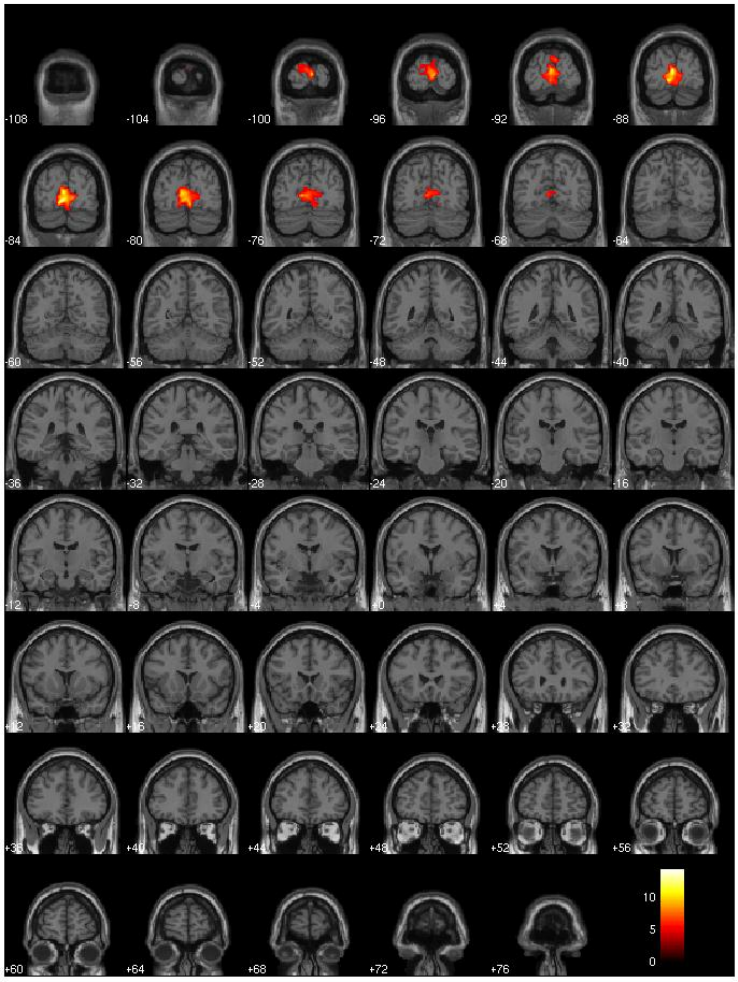
Clusters Of Brain Activation In Non-Autism Group During Neutral Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_=$)	x	y	z
Left calcarine	<0.001	2158	5.00	-4	-84	-2

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 9

Clusters Of Brain Activation In Non-Autism Group During Neutral Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Autism group

The Autism group showed a large region of significant activation, again with a peak co-ordinate in the left calcarine sulcus, and extending to bilateral calcarine sulci, lingual gyri, cuneus, fusiform gyri and cerebellum lobule VI.

Table 6

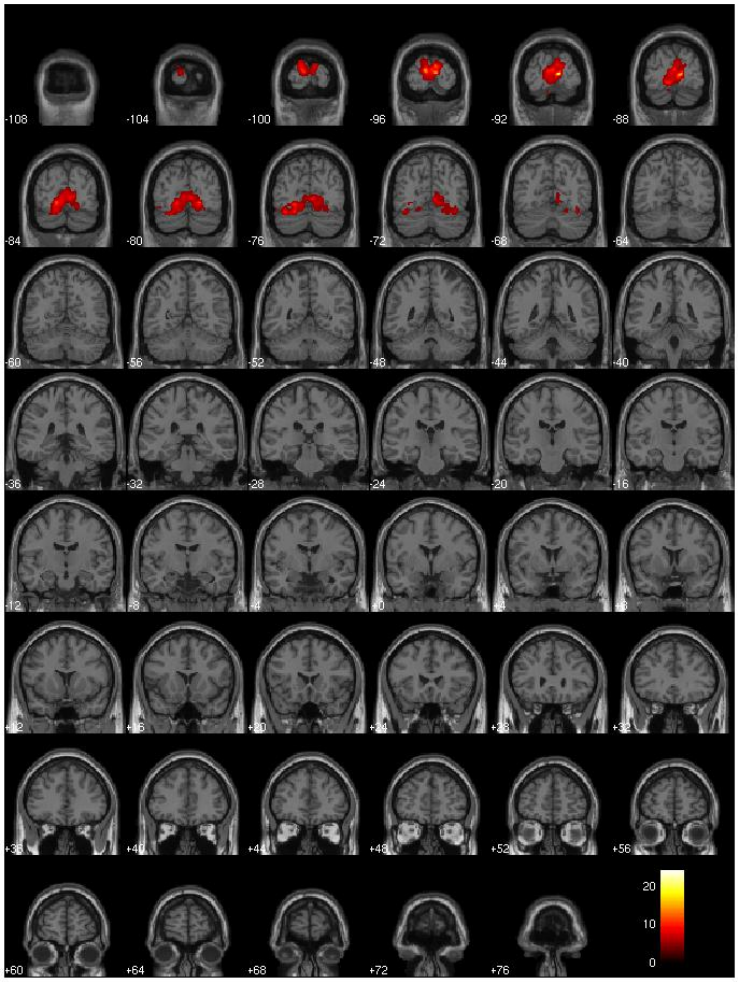
Clusters Of Brain Activation In Autism Group During The Neutral Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left calcarine	<0.001	3616	5.75	10	-90	4

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 10

Clusters Of Brain Activation in Autism Group During The Neutral Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Contrast: fear > baseline

Non- Autism group

The non-Autism group showed a large region of significant activation with a peak co-ordinate in the left calcarine sulcus, and extending to the left fusiform gyrus and bilateral calcarine sulci, lingual gyri and cuneus. A second, smaller, cluster was also identified with a peak co-ordinate in the left cerebellum extending to the left fusiform gyrus.

Table 7

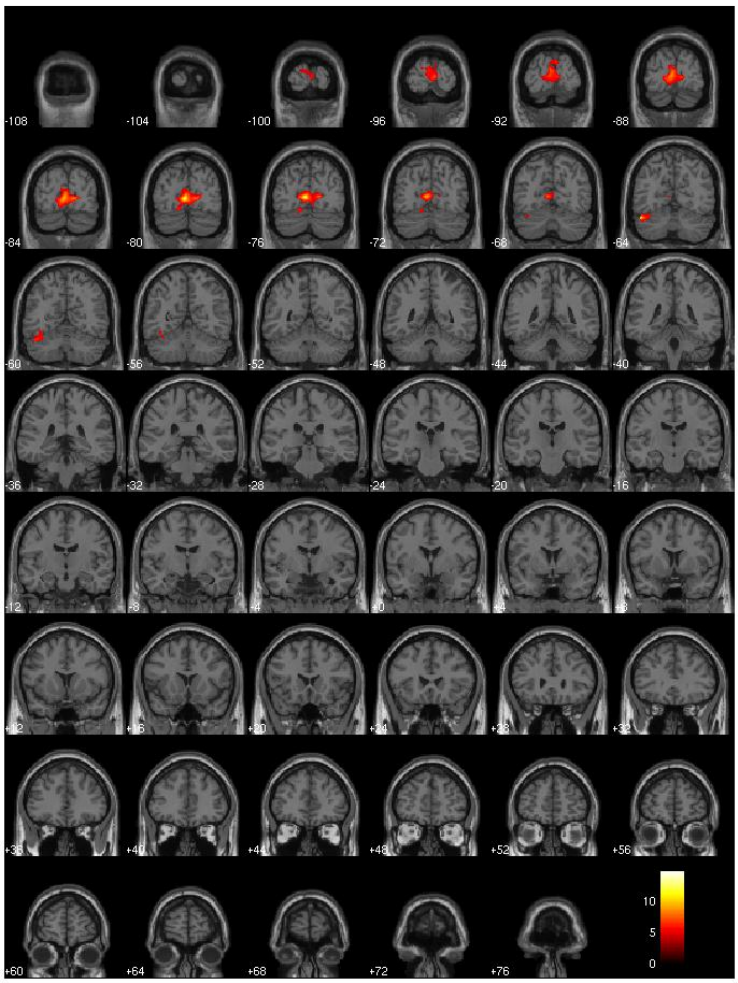
Clusters Of Brain Activation In Non- Autism Group During The Fearful Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{=}$)	x	y	z
Left calcarine	<0.001	1662	5.05	-4	-78	4
Left cerebellum	0.015	160	4.67	-48	-64	-26

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 11

Clusters Of Brain Activation In Non- Autism Group During The Fearful Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Autism group

The Autism group showed a large region of significant activation with a peak co-ordinate in the right lingual gyrus, and extending to bilateral lingual gyri, calcarine, fusiform gyri and cuneus.

Table 8

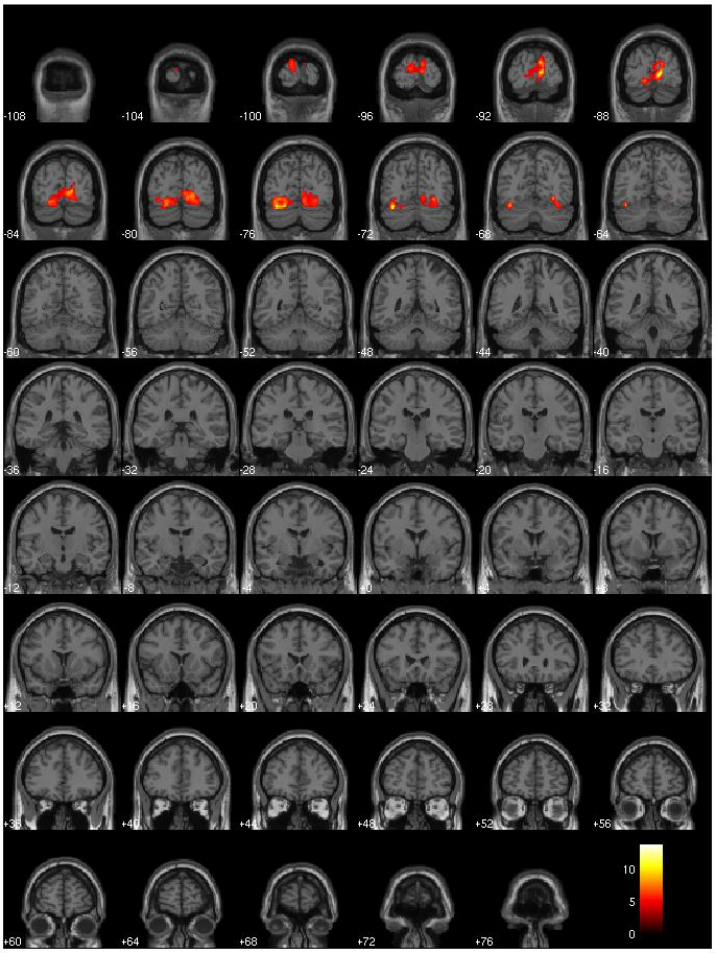
Clusters Of Brain Activation In The Autism Group During The Fearful Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Right lingual gyrus	<0.001	2398	4.95	-34	-74	-20

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 12

Clusters Of Brain Activation In The Autism Group During The Fearful Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Contrast: neutral faces > fearful faces

Non-Autism group

The non-Autism showed no clusters of significant brain activation to this contrast.

Autism group

The Autism group showed a cluster of significant activation with a peak co-ordinate in the left superior frontal gyrus.

Table 9

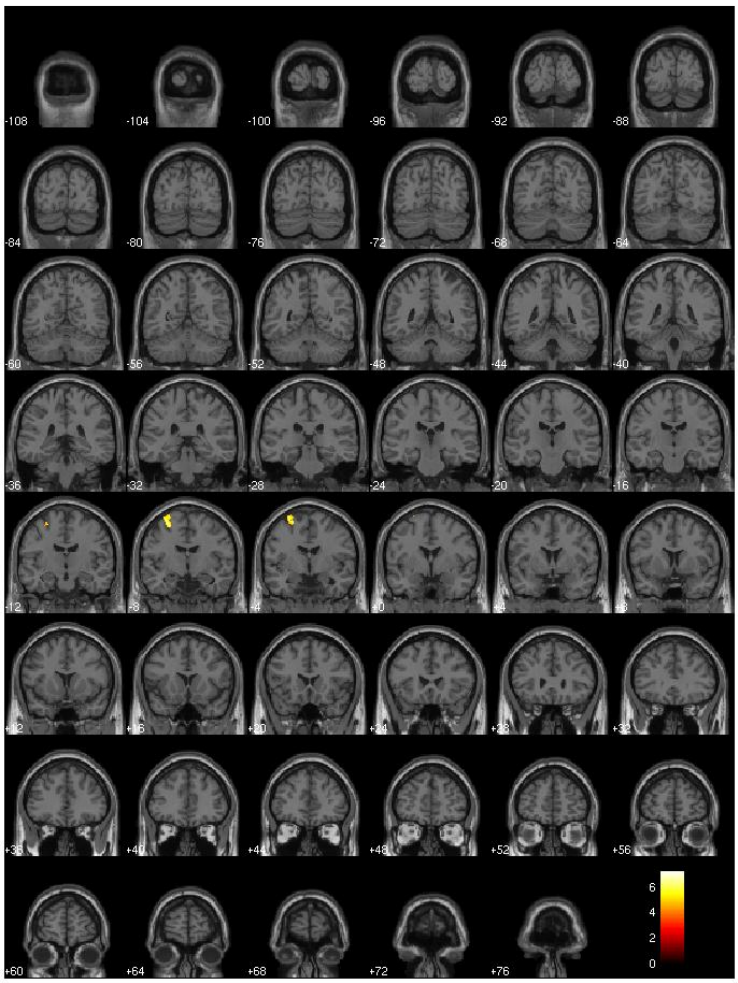
Clusters Of Brain Activation In The Autism Group During The Neutral Faces Versus Fearful Faces Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left superior frontal gyrus	0.022	125	3.91	-26	-6	62

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 13

Clusters Of Brain Activation In The Autism Group During The Neutral Faces Versus Fearful Faces Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Contrast: fearful faces > neutral faces

There were no regions of significant brain activation identified for the reverse contrast of fearful faces versus neutral faces in either group.

Between group results

In the between group contrasts, clusters of significantly different activation between the groups are shown for each contrast.

Contrast: neutral faces > baseline

The non-autism group showed no clusters of significantly greater activation than the autism group in the neutral faces versus baseline contrast. The Autism group showed one cluster of significantly greater activation than the non-autism group in the same contrast; with a peak co-ordinate in the right precentral gyrus and extending to the postcentral gyrus and the rolandic operculum (part of the insula cortex). A second cluster showed a trend towards significance with a peak co-ordinate in the left precentral gyrus.

Autism > Non- Autism

Table 10

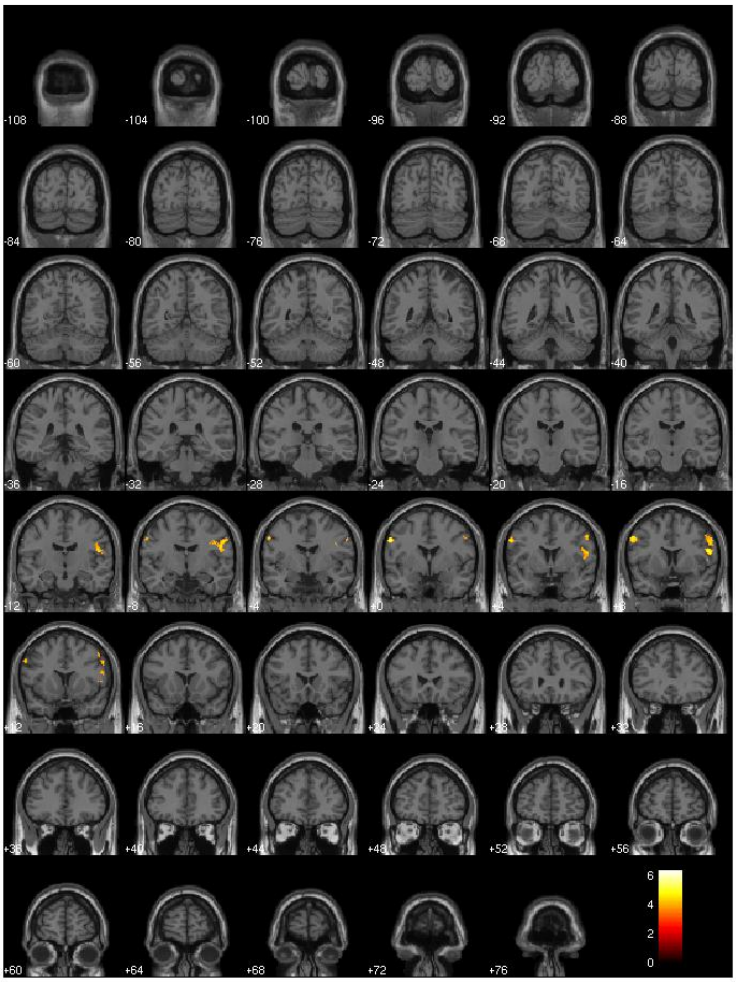
Clusters Of Greater Brain Activation In The Autism Group Compared To The Non-Autism Group During The Neutral Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Right precentral gyrus	0.001	385	4.15	56	8	18
Left precentral gyrus	0.096	145	4.43	-56	0	34

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 14

Clusters Of Greater Brain Activation In The Autism Group Compared To The Non-Autism Group During The Neutral Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Contrast: fearful faces > baseline

The non-autism group showed two clusters of significantly greater activation than the autism group in the fearful faces versus baseline contrast; one cluster including the left superior frontal gyrus, and the other including the left angular gyrus, left supramarginal gyrus and left inferior parietal lobule. The autism group showed no clusters of significantly greater activation in the same contrast.

Non-Autism > Autism

Table 11

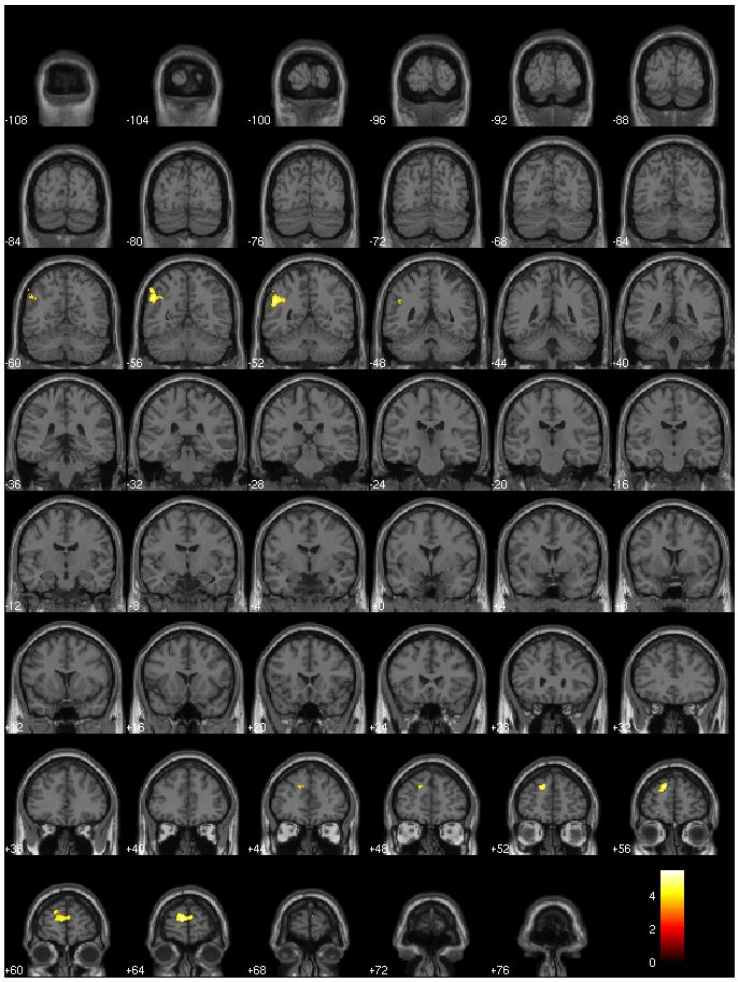
Clusters Of Greater Brain Activation In The Non-Autism Group Compared To The Autism Group During The Fearful Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left superior frontal gyrus	0.006	282	3.97	-12	56	30
Left angular gyrus	0.017	231	4.09	-50	-54	32

Note. Only clusters of $p<0.1$ family-wise error-corrected significance are reported.

Figure 15

Clusters Of Greater Brain Activation In The Non-Autism Group Compared To The Autism Group During The Fearful Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Contrasts comparing activations to neutral and fearful faces

Neither group showed any clusters of significantly different activation to the more subtle contrasts of fearful faces versus neutral faces or the reverse, even at the lower statistical threshold.

Small volume corrections

The Activation Likelihood Estimation (ALE) meta-analysis by Philip (2012), reported clusters of significantly greater likelihood activations in autistic subjects compared to non-autistic subjects. For these small volume correction (SVC) analyses, masks were made for both of the contrasts: autism > non- autism and non-autism > autism using the clusters reported on the meta-analysis of basic social tasks (which included tasks of face/emotion processing). Each mask included a series of individual spheres was created, each centred on the co-ordinates from the meta-analysis and of a radius so as to be of the same volume as the area reported in the meta-analysis. These masks were then applied to the previous analyses. For the SVC analyses, the results were thresholded at 0.001, with results that are family-wise error-corrected $p<0.05$ reported. Table 12 shows the regions from the Philip et al meta-analysis of basic social tasks that were used to create the mask for the SVC analyses.

Table 12

Regions identified in Philip et al (2012) ALE meta-analysis used to create mask for SVC analyses.

Comparison, region	Voxels	x	y	z
ASD > Control				
Left superior temporal gyrus	984	-64	-28	0
Right superior temporal gyrus	360	57	11	0

Control > ASD				
Left Fusiform Gyrus	2424	-20	-90	-22
Right Inferior Occipital Gyrus	1080	29	-85	-22-
Right Culmen	248	42	-40	31
Left Middle Temporal Gyrus	248	-56	-40	-6

One significant result was found, within the autism vs non-autism comparison of the neutral faces>baseline contrast in the right rolandic operculum, highlighting an overlap between results in the analysis of the present study and those reported in the ALE meta-analysis. The results of this are shown in Table 13.

Table 13

Clusters Of Greater Brain Activation In The Autism Group Compared To The Non-Autism Group During The Neutral Faces Versus Baseline Contrast Using A Small Volume Correction For Results From Philip et al (2012) ALE Meta-analysis.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Right rolandic operculum	0.022	13	3.42	54	8	6

Note. Only clusters of $p<0.1$ family-wise error-corrected significance are reported.

Regression analyses

Within the autism group, regression analyses were completed for each of the main contrasts, regressing activation against ADOS Calibrated Severity Scores (CSS). Results were thresholded at $P<0.001$ uncorrected, with only results being significant at $p<0.1$ family-wise error-corrected shown.

No significant correlations were found in any of the contrasts examined.

Discussion

This study set out to examine the question of whether, in a group with low average cognitive ability, autistic individuals show different patterns of brain activation on a functional imaging paradigm exploring response to processing emotional facial stimuli.

Foremost, whilst all the participants had had previous structural scans, this was their first functional scan. That the experience and task was acceptable to the participants and that the expected activations were seen in the non-autism group validates the use of the task in this population previously largely excluded from imaging research.

In keeping with the hypothesis, a number of differences were found between the non-autism group and the autism group; some of which overlapped the findings of a previous meta-analysis of functional imaging in autism.

It had been hypothesised that the principal finding would be of a reduced neural response to emotional (fearful) faces in the autistic group; and indeed this was borne out in the between-group comparison of response to fearful faces versus baseline. However, perhaps the more interesting finding was that of enhanced response in the autistic group to neutral facial stimuli.

Response to neutral facial stimuli

In both the autistic and non-autistic groups, large clusters of activation were seen in response to the neutral faces versus baseline contrast. These clusters, as expected, included large posterior regions including bilateral lingual gyri and cuneus. It is interesting to note that the patterns of activation appear to be more diffuse in the autistic group than in the non-autistic group, something previously reported in autistic participants of average cognitive ability (R. C. M. Philip, 2009).

In the more subtle contrast comparing differential response to neutral and fearful stimuli, the autistic group showed a cluster in the left superior frontal gyrus of increased activation in the neutral faces>fearful faces contrast. This suggests that neutral stimuli may in some way be evoking a greater response than fearful stimuli in these regions. The mechanisms that underlie this are not clear, however, it has previously been reported that autistic individuals may perform more poorly on discriminating ambiguous stimuli (Law Smith, Montagne, Perrett, Gill, & Gallagher, 2010; Wong, Beidel, Sarver, & Sims, 2012) and it is possible that this hyper-activation represents either difficulty in interpreting a neutral stimulus, or perhaps a tendency to interpret neutral stimuli as negative (Eack, Mazefsky, & Minshew, 2015). Interestingly, a similarly inversed pattern of increased activation to neutral faces (compared to fearful faces) has also been previously reported in children (Thomas et al., 2001); raising the possibility that this pattern may reflect a developmental stage.

In the between-group analysis comparing the response to neutral faces versus baseline there were no clusters of significantly greater activation in the non-autistic group; however, the autistic group had one cluster of significantly greater activation than the non-autistic group. This cluster of significantly greater activation in the right prefrontal gyrus / inferior frontal gyrus was also mirrored by another cluster of a trend towards significance in the left prefrontal gyrus / inferior frontal gyrus. In general, the IFG has more typically been associated with relative hypo-activation to faces (Malisza, 2011) and also specifically to neutral faces in ASD (Bookheimer, Wang, Scott, Sigman, & Dapretto, 2008; Hadjikhani et al., 2007; Koshino et al., 2008). However, hyper-activation in autistic individuals has also been reported, mainly when considering response to non-facial stimuli (e.g. arrows or objects) (Greene et al., 2011; Vaidya et al., 2011). This said, the small volume correction analysis confirmed that the cluster on the right side overlapped a cluster reported in Philip et al (2012) in the right rolandic operculum. This particular part of the rolandic operculum is an area previously described as having mirror neurons; differential activation of which has been implicated in both emotion processing and autism. It is of interest that this is a region, in which differential activation appears to be important in autism; is highlighted across individuals of varying cognitive ability.

As with all imaging studies, trying to establish what this hyper-activation represents is far from straightforward. This increased activation could be

associated with diminished, similar, or enhanced processing of the neutral stimuli. The finding of generally similar performance of autistic participants on accuracy in identifying neutral faces in previous studies (Kleinhans et al., 2009; Koshino et al., 2008; Pierce et al., 2001; Schultz et al., 2000) potentially supports the idea that this increased activation may occurs in the context of similar performance. At the risk of attributing function too closely to structure, it may be that these clusters of increased activation in autistic individuals associated with neutral faces represent excess neural activity in the face of ambiguous stimuli, which ultimately may not be associated with any difference in performance.

There is a growing literature around the phenomenon of 'camouflaging' in autistic individuals; that is characterised by, "using explicit techniques to appear socially competent, and finding ways to prevent others from seeing their social difficulties" (L. Hull et al., 2017). In some of the descriptions of camouflaging, autistic individuals describe the effort that social interactions take; and how it can be, "very draining trying to figure out everything all the time" (Bargiela, Steward, & Mandy, 2016). It is far from clear that the finding of increased activation to neutral stimuli in the autistic group is linked to this phenomenon; however, there are potential interesting parallels to be drawn. If common social situations are seen as potentially ambiguous and necessitate conscious effort to understand, then it's possible that the neutral faces paradigm studied here may represent a model for this phenomenon. Whilst autistic individuals have typically been described as having diminished theory of

mind or ability to mentalise; some of the narratives of autistic individuals are now suggesting that whilst there may be difficulties in some of these skills, it is perhaps underpinned by an experience of 'hyper-mentalising' with increased effort being brought to bear in trying to understand and act on social situations (Bargiela et al., 2016). Potentially, the clearer, and simple, fearful facial stimuli require less resource to process; explaining the lack of the same effect in that contrast.

Response to fearful facial stimuli

In the emotional (fearful) faces versus baseline contrast both groups showed significant clusters of activation around bilateral calcarine sulci, extending to fusiform and lingual gyri, and cuneus. As with the previous contrast examining response to neutral faces, the activation in the autistic group appeared to have a more diffuse pattern.

Considering the between-group analysis on the fearful faces to baseline contrast, the autistic group showed no clusters of increased activation compared to the non-autistic group. However, the non-autistic group showed two clusters of significantly greater activation on this contrast; one centred in the left superior frontal gyrus, and one spanning the left angular gyrus, left supramarginal gyrus and left inferior parietal lobule. This result is in keeping with the original hypothesis that the non-autistic group would show increased activations to fear compared to the autistic group. The result in the left angular gyrus is the same region identified by Philip (2009) in their functional imaging

study of autistic individuals of average cognitive ability using the same paradigms. In their study, they reported two clusters of significantly greater activation on a fearful faces to neutral faces contrast, with typically-developing controls showing greater activations in the right inferior parietal lobe and the left inferior parietal lobe/angular gyrus. This adds weight to the finding; suggesting that at least in this instance, the finding has some translatability across groups of different cognitive ability.

Conclusions

In this study there were two main findings. Firstly, clusters of significantly greater activation were found in a group of non-autistic individuals compared to an age- and IQ-matched autistic group on an fMRI task examining response to fearful facial stimuli. This finding is in keeping with the literature, showing that autistic individuals do not appear to have the same pattern of response to emotional facial stimuli.

The other finding of the study, which is potentially of greater interest, is that on three different analyses autism was associated with significantly greater activations to neutral facial stimuli. The mechanisms underpinning this are yet to be elucidated, however, the potential that this links in with the descriptions by autistic individuals of increased effort to understand social situations, and that the neutral faces paradigm perhaps is a model for ambiguous social cues is interesting. Further study could usefully consider this and integrate in-scanner analyses with participant narratives of their experience both of real-life social situations, as well as responses to the paradigms in use.

Chapter 6: A functional imaging study of autism in individuals with fragile X syndrome

Introduction

This chapter presents results from the imaging analyses of the individuals with fragile X syndrome. Each of the contrasts is considered both within the autism and non-autism groups as well as between the groups. Further analyses using a small volume correction and regression analyses are also included. The results of a further omnibus analysis considering the combined results of both the SEN and FXS cohorts are then presented.

Participant details

The baseline details of participants included in the imaging analyses are shown in Table 14.

Table 14

Baseline Characteristics Of Autism And Non-Autism Fragile X Syndrome Groups

	Autism group	Non-Autism group
N	10	7
Male : female	8:2	5:2
Age	18 (6.2)	27 (11.7)
Full-scale IQ	59 (8.9)	63 (14.2)
Verbal IQ	69 (11.9)	71 (14.2)
Performance IQ	58 (11.1)	60 (14.4)
ADOS Total	15 (10-20)	2 (0-5)
ADOS CSS	8 (6-10)	2 (1-2)

Note. Results show group means (s.d.) for age and IQ; and median (range) for the ADOS scores. The groups were not significantly different on gender (Chi square = 0.168, p=0.682), age (p = 0.092), full-scale IQ (p = 0.528), verbal IQ (p = 0.695) or performance IQ (p = 0.704) (independent samples t-tests). ADOS, Autism Diagnostic Observation Schedule; CSS, Calibrated Severity Score.

In general, the use of prescription medication in the participants was low, with only four participants taking regular psychoactive medication. All four participants were taking mavoglurant (AFQ056, Novartis); one in the non-autism group and three in the autism group. The relatively low use of psychoactive medications in the sample is likely to represent a combination of prescribing practice in the U.K. and a degree of selection bias; in that those who were more affected and thus more likely to be on medication, were less likely to be able to participate. Further, the sample was on average older than those in most prior studies. As such, whilst a number of participants had been on psychoactive medications as children; they were no longer. Epilepsy, whilst more common in FXS, was under-represented in this sample; with no participants being treated for epilepsy.

Statistical analyses used

For the imaging analyses, one-sample t-tests were used for within group results, and 2-sample t-tests were used for the between group analyses, with age included as a covariate. All results were thresholded at 0.001, with results reported as family-wise error, cluster-corrected p-values. Results reported include all those with $p < 0.1$; with family-wise error-corrected $p < 0.05$ being considered significant and $0.100 > p > 0.05$ being considered a non-significant trend. For each cluster, the significance level ($p_{FWE\text{-}corr}$), cluster extent in voxels (k_E), z-score ($Z\equiv$), and MNI co-ordinates (x, y, z) are given for the peak voxel in the cluster. Regions annotated were identified using the Automated Anatomical

Labelling Atlas 3 (Rolls et al., 2019) running in SPM12. The results for the all faces > baseline contrasts, as secondary analyses, are included in Appendix 6.

Results

Within group results

Contrast: neutral faces > baseline

This contrast shows the regions of brain activity associated with viewing the neutral faces in contrast to baseline.

Non-autism group

The non-autism group showed a large region of significant activation with a peak co-ordinate in the left calcarine sulcus and extending to bilateral calcarine sulci, right cuneus and left and right middle and superior occipital gyri.

Table 15

Clusters of brain activation in non-autism group during neutral faces versus baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left calcarine	0.001	402	4.68	0	-100	-6

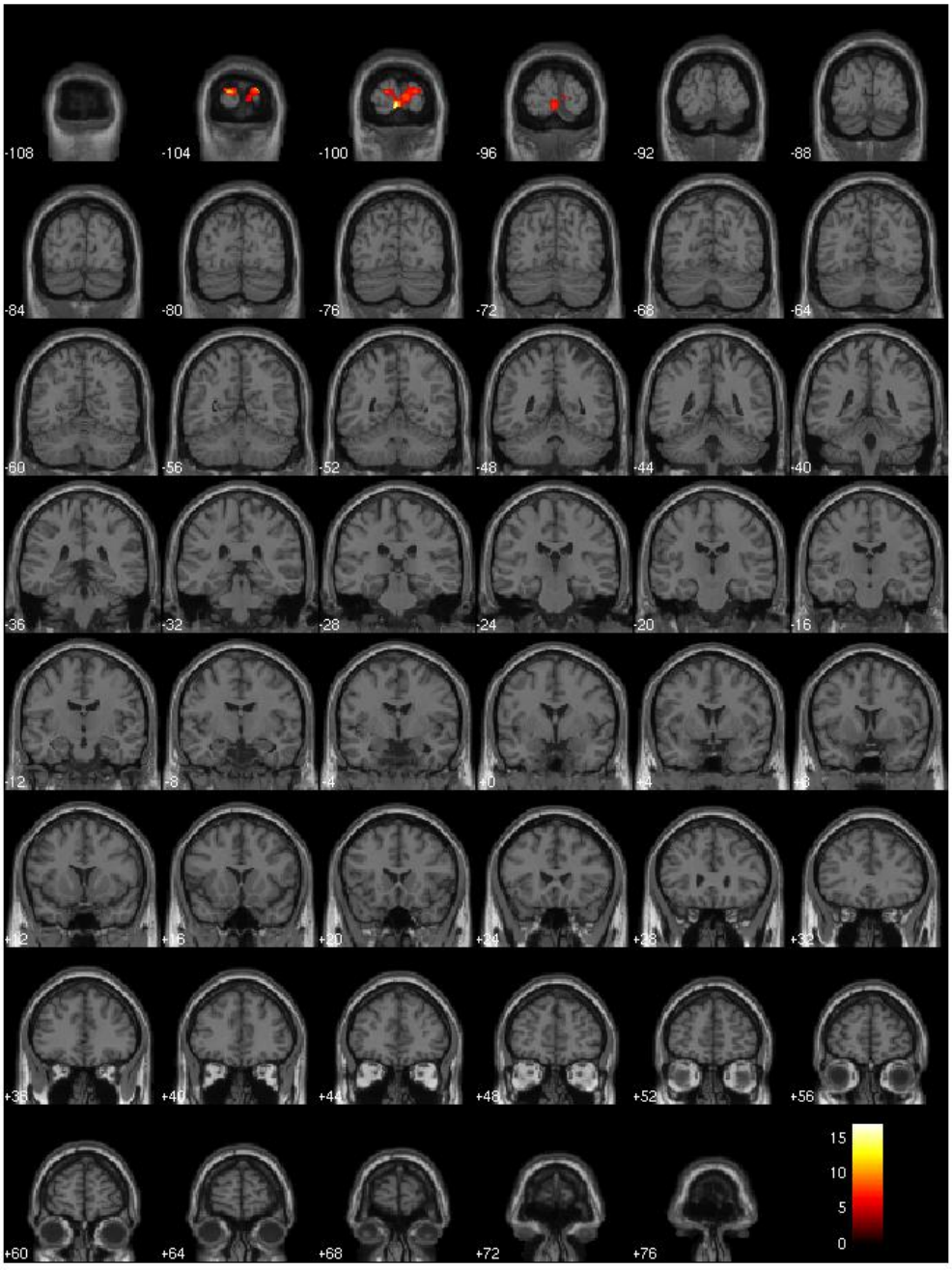
Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Autism group

The autism group showed no regions of significant activation during the neutral faces versus baseline contrast.

Figure 16

Clusters of brain activation in non-autism group during neutral faces versus baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Contrast: fearful faces > baseline

Non-autism group

The non-autism group showed a large region of significant activation with a peak co-ordinate in the left calcarine sulcus and extending to include bilateral calcarine sulci, bilateral cuneus and the right lingual gyrus. A second, smaller, cluster was also identified with a peak co-ordinate in the left ventral striatum and extending to the left rectus and left caudate. A third cluster was also identified with a peak co-ordinate in the left precentral gyrus.

Table 16

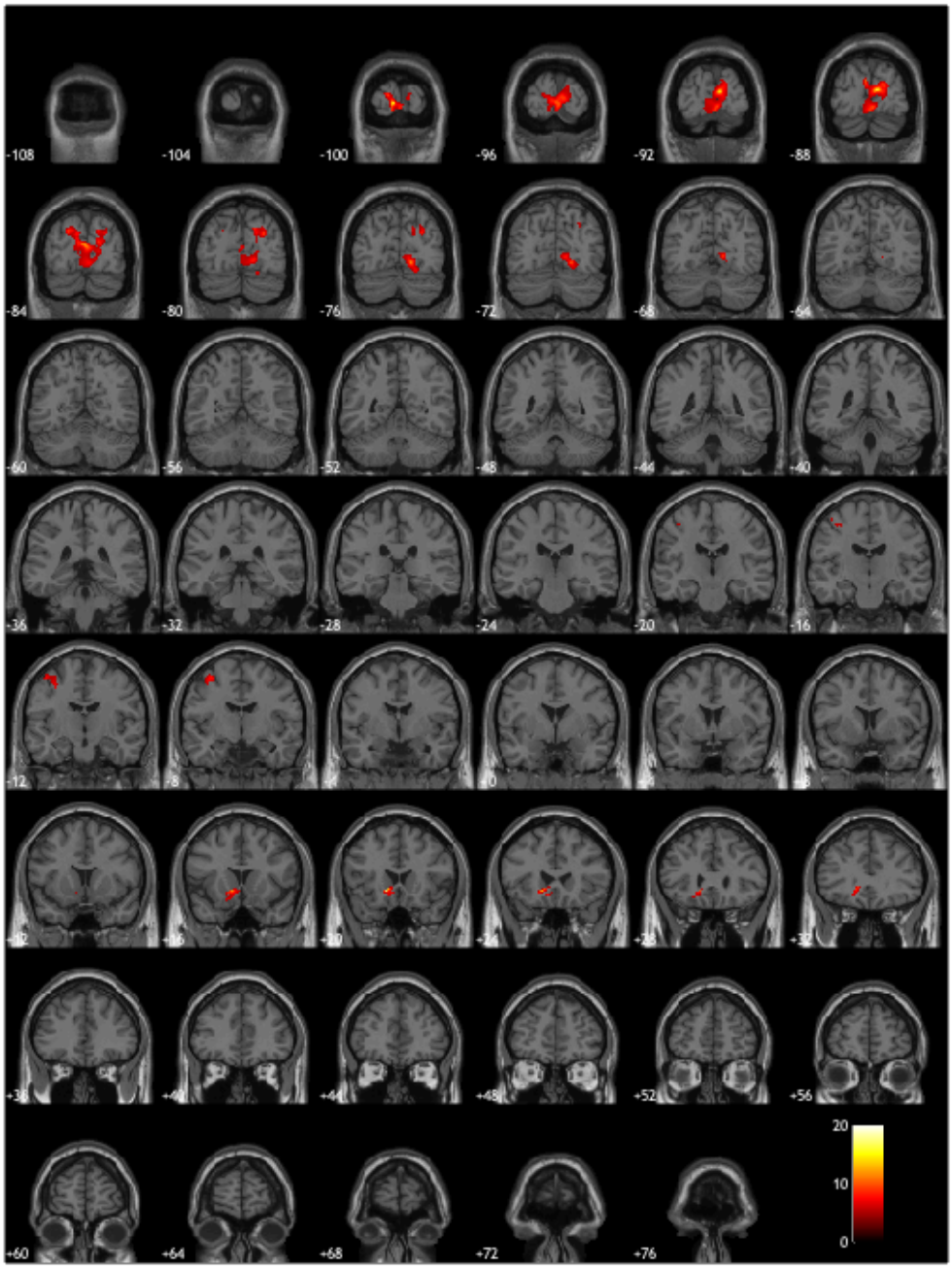
Clusters of brain activation in non-autism group during the fearful faces versus baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left calcarine	<0.001	1962	4.64	10	-90	12
Left ventral striatum	0.004	199	4.90	-16	22	-6
Left precentral gyrus	0.037	128	4.11	-36	-6	56

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 17

Clusters of brain activation in non-autism group during the fearful faces versus baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Autism group

The autism group showed a large region of significant activation, with a peak co-ordinate in the left calcarine sulcus, and extending to bilateral calcarine sulci and cuneus, and left middle and superior occipital gyri. A second, smaller region of activation was also found with a peak co-ordinate in the left supplementary motor area and extending to bilateral supplementary motor areas.

Table 17

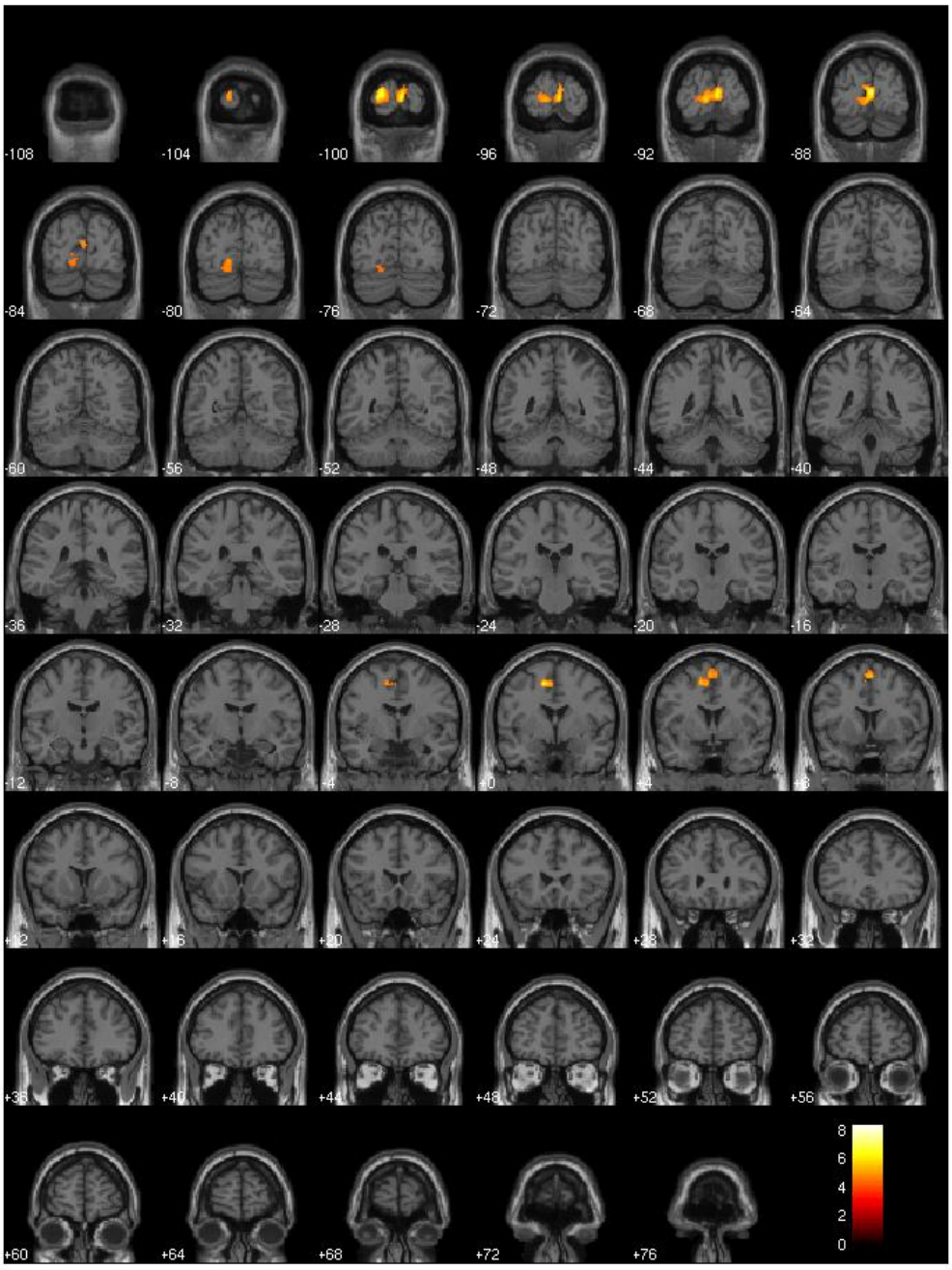
Clusters of brain activation in the autism group during the fearful faces versus baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left calcarine	<0.001	889	8.35	-16	-102	6
Left supplementary motor area	0.037	221	7.55	-12	2	52

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 18

Clusters of brain activation in the autism group during the fearful faces versus baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Between group analyses

Contrasts were run for each of the 4 main contrasts (fear > baseline, neutral > baseline, fear > neutral, neutral > fear), for both the autism > non-autism contrast, and the corresponding non-autism > autism contrast, including age as a covariate.

On the non-autism > autism group comparison of the fear > baseline contrast, a region of significant difference was found in the left superior temporal gyrus extending to the left supramarginal gyrus and left rolandic operculum. The cluster remains significant ($p_{FWE\text{-}corr} = 0.002$; $k_E = 511$; $(Z_{\equiv}) = 4.52$; x,y,z = -64, -30, 22) when including medication use as a covariate.

A further cluster at the level of a non-significant trend towards difference was also found in the left cuneus, extending to the left precuneus and left superior occipital gyrus.

Table 18

Clusters of significantly greater brain activation in the non-autism group compared to the autism group during the fearful faces versus baseline contrast.

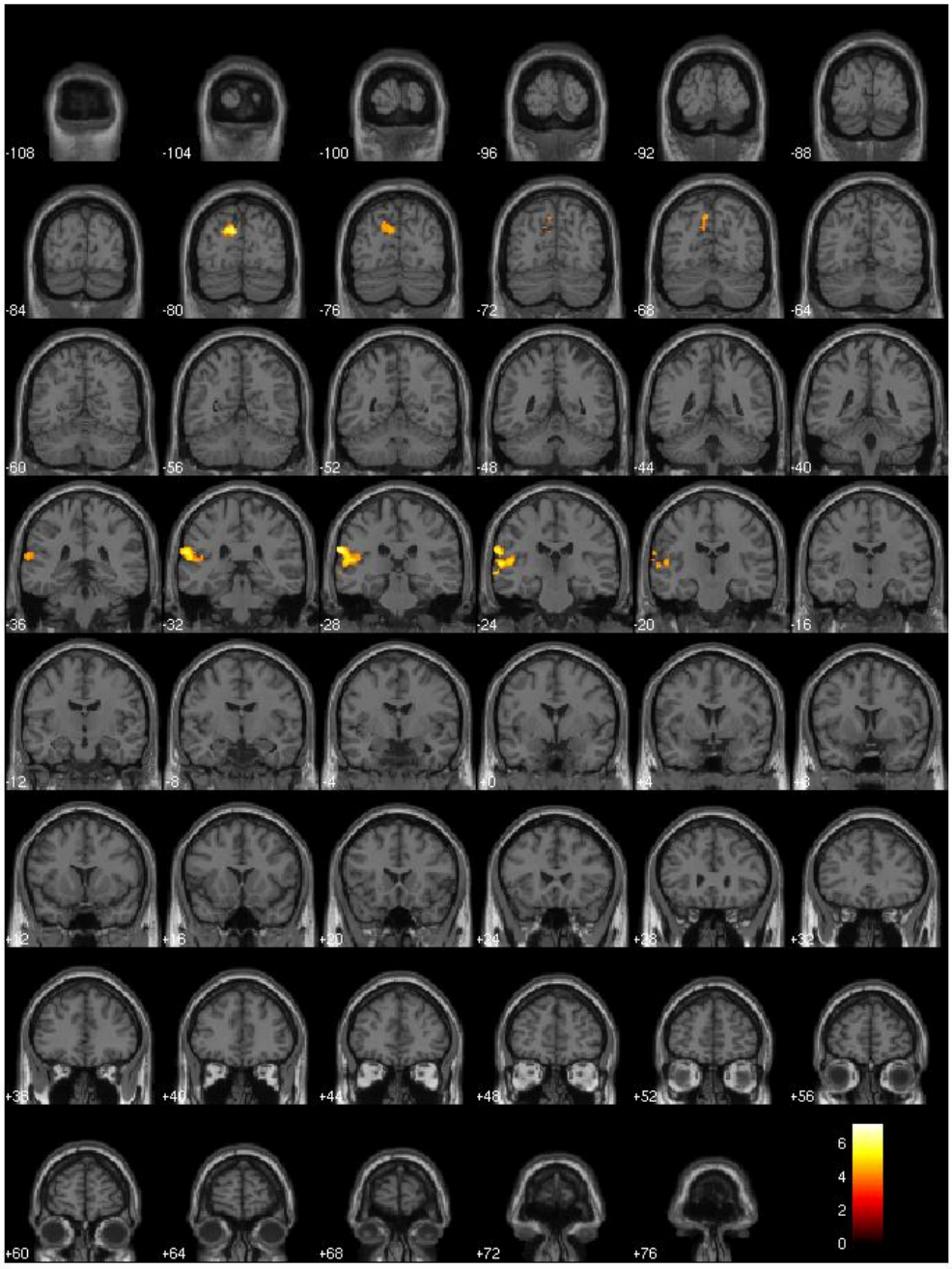
Cluster	$p_{FWE\text{-}corr}$	k_E	$(Z_{\equiv})$	x	y	z
Left superior temporal gyrus	0.001	570	4.45	-64	-30	22
Left cuneus	0.065	223	4.56	-8	-80	28

Note. Co-ordinates are given for the peak voxel in the cluster in MNI space. Only clusters of p<0.1 family-wise error-corrected significance are reported.

No other significant clusters were seen on any of the remaining between group contrasts.

Figure 19

Clusters of significantly greater brain activation in the non-autism group compared to the autism group during the fearful faces versus baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Small volume corrections

As in the previous chapter, for these small volume correction (SVC) analyses, masks were created using the reported clusters of significantly more likely activation on basic social tasks from Philip (2012) for both of the contrasts: autism > non- autism and non-autism > autism. Each mask included a series of individual spheres was created, each centred on the co-ordinates from the meta-analysis and of a radius so as to be of the same volume as the area reported in the meta-analysis. These masks were then used to re-run the analyses. For the SVC analyses, the results were thresholded at 0.001, with results that are family-wise error-corrected $p<0.05$ reported.

The mask used was based on the same regions as reported in Table 12 in chapter 4.

No significant results were found within any of the contrasts.

Regression analyses

Regression analyses were completed for each of the main contrasts, regressing activation against ADOS Calibrated Severity Scores (CSS) in the autism group. Results were thresholded at $P<0.001$, with only results significant at $p<0.1$ family-wise error-corrected shown.

There were no significant correlations for any of the contrasts.

There was, however, a significant positive correlation between autism CSS score and activation to the faces > baseline contrast in a cluster in the left cerebellum, including lobules IV, V and VI; the results of which are in Appendix 6.

Exploring differences between the special education needs and fragile X syndrome groups

In these analyses, the SEN and FXS groups are analysed together to consider whether there are any differences between the groups as a whole. As noted in the materials and methods chapter, the SEN cohort data was re-pre-processed using the ArtRepair pipeline used for the FXS group data so as to make the data comparable for these analyses. Age, IQ and number of trigger activations were used as covariates. The baseline characteristics of the two groups are shown in Table 19 below.

Table 19

Baseline characteristics of the SEN and FXS groups

	SEN group	FXS group
N	18	17
Male : female	15 : 3	13 : 4
Age	24.3 (1.9, 22-28)	21.9 (9.7, 12 – 46)
Full-scale IQ	69.7 (13.4)	60.9 (11.1)
Verbal IQ	69.9 (14.9)	69.7 (12.6)
Performance IQ	74.5 (11.5)	58.9 (12.2)
Autism : Non-Autism	9:9	10:7
ADOS Total*	7 (0-16)	11 (0-20)
ADOS CSS*	3.5 (1-9)	6 (1-10)

Note. Results show group means (s.d.) for age and IQ; and median (range) for the ADOS scores.
*ADOS data only available for 16 of 18 SEN participants.
ADOS, Autism Diagnostic Observation Schedule

Contrast: neutral faces > baseline

There were no significant results on this contrast considering the effect of group (FXS vs SEN).

Contrast: fearful faces > baseline

In this contrast, two clusters (one significant, and one a trend towards significance) with peak co-ordinates in the left and right supplementary motor areas, both extending to middle cingulate, were found in the FXS > SEN contrast. In contrast, there were no significant results found in the SEN > FXS contrast.

Table 20

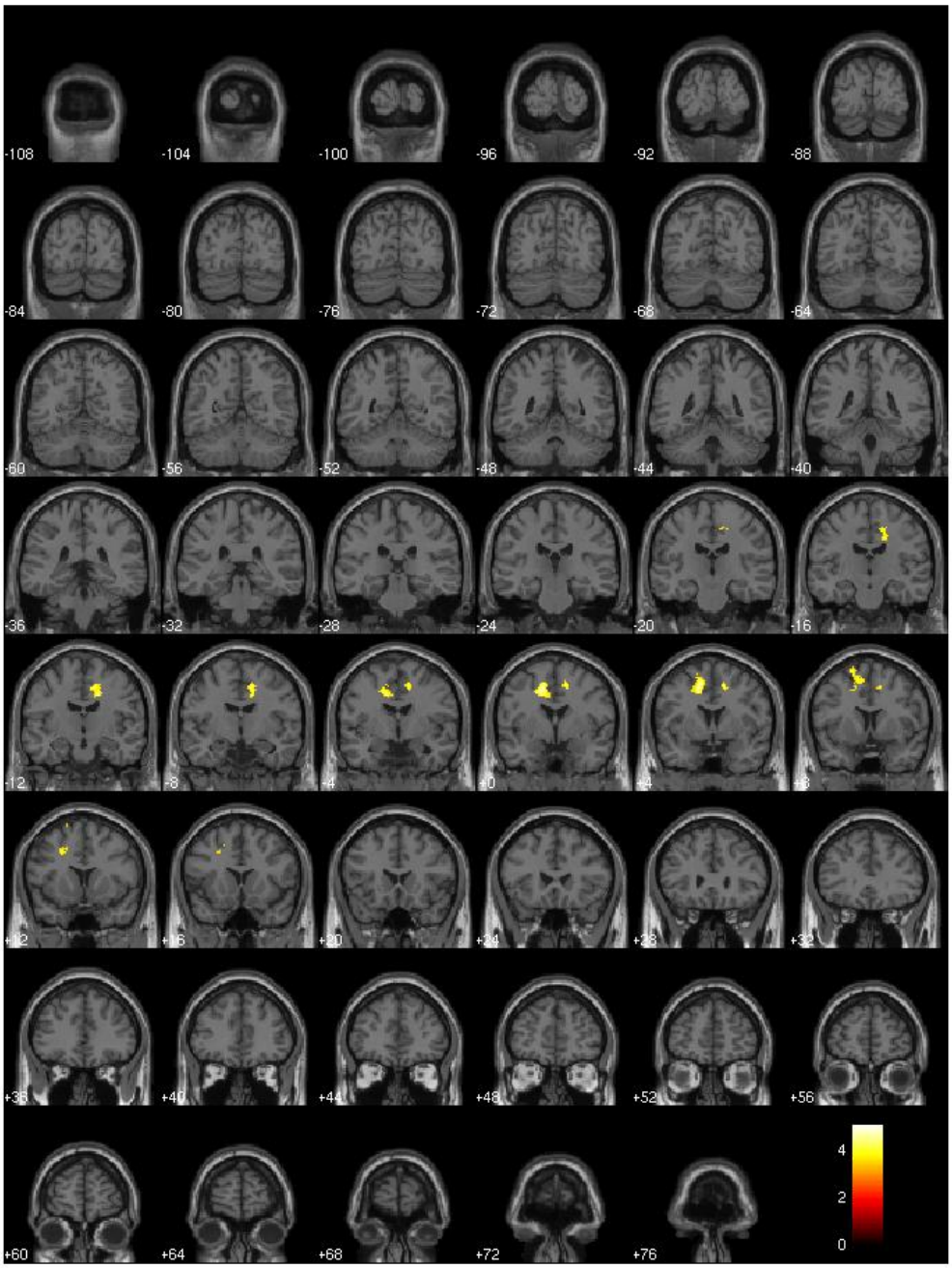
Clusters of significantly greater brain activation in the FXS group compared to the SEN group during the fearful faces versus baseline contrast

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left supplementary motor area	0.019	388	4.20	-14	0	42
Right supplementary motor area	0.085	245	3.76	14	-6	48

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 20

Clusters of significantly greater activation in the FXS group compared to the SEN group on the fear > baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Interaction of group status and autism status

When examining the interaction of autism status and group status on the fearful faces > baseline contrast, one significant result emerged with a peak co-ordinate in the left superior temporal gyrus, extending to the left supramarginal gyrus and left rolandic operculum, as shown in Table 21 and Figure 21.

Table 21

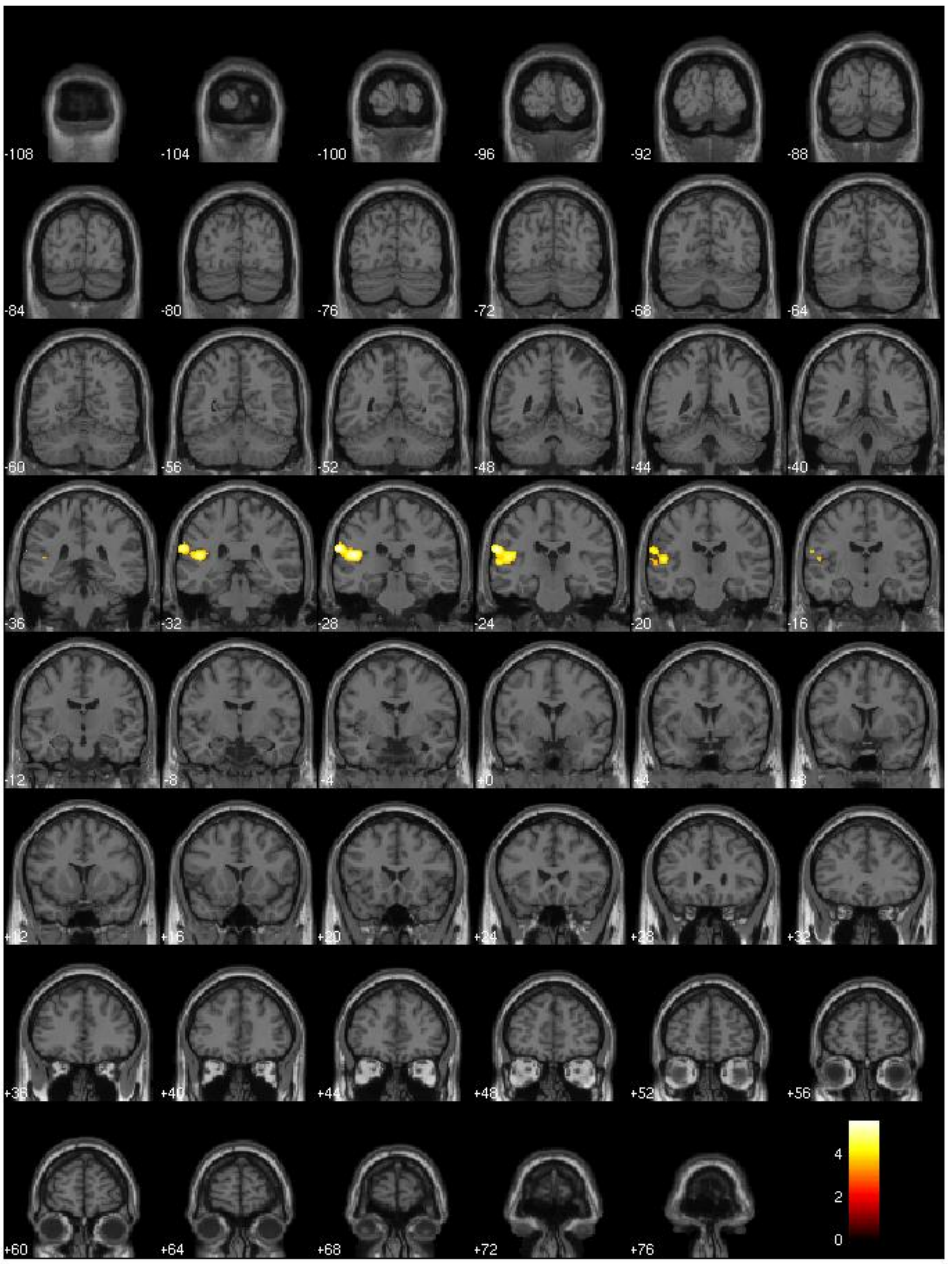
Cluster of significantly different activation in the positive interaction of SEN vs FXS x Autism vs non-Autism groups on the fear > baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left superior temporal gyrus	0.001	730	4.52	-64	-26	24

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure 21

Clusters of significantly different activation in the contrast exploring the positive interaction between SEN vs FXS x Autism vs non-Autism groups on the fear > baseline contrast.



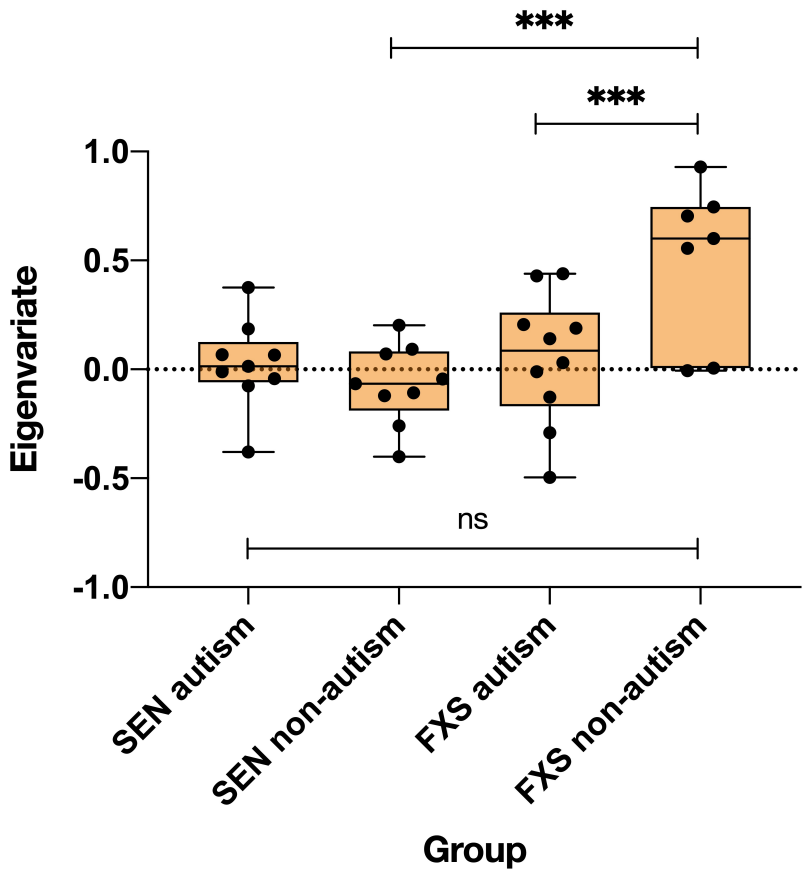
Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Exploring the interaction finding

Eigenvalues were extracted for the cluster identified in the positive interaction finding on the fear versus baseline contrast to explore the result in left superior temporal gyrus (-64 -26 24). These are plotted below in Figure 22.

Figure 22

Eigenvalues from cluster in the left superior temporal gyrus (-64 -26 24) from the fear versus baseline contrast for all four groups



Note. *** denotes p<0.001. SEN; Special Educational Needs, FXS; Fragile X Syndrome. Contrasts of FXS non-autism vs FXS autism and FXS non-autism vs SEN non-autism shown in tables 22 and 23.

Table 22

Cluster of significantly greater activation in the FXS non-autism group compared to the FXS autism group on the fear > baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left superior temporal gyrus	<0.001	1276	5.20	-64	-30	24

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Table 23

Cluster of significantly greater activation in the FXS non-autism group compared to the SEN non-autism group on the fear > baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left superior temporal gyrus	<0.001	900	4.76	-64	-30	20

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Discussion

This study explored the role of autism in facial emotion-processing in individuals with fragile X syndrome; and particularly whether there were parallels in the findings between previous research in the autism or FXS imaging literature.

Previous functional imaging studies of facial emotion processing in both autism and FXS had suggested that differences in activation in the fusiform face area and the superior temporal gyrus were some of the most robust findings. Interestingly, whilst the within-group analyses showed fusiform activation, there were not any differences between the groups in this region.

Response to neutral facial stimuli

Whereas in the SEN imaging cohort, where there had been a group difference on response to neutral facial stimuli (with the autistic group showing a cluster of significantly increased activation), there was no such finding in the FXS group. Instead, the results were somewhat more ambiguous. In the within-group analyses, the FXS non-autism group had shown a cluster of significant activation to the neutral facial stimuli compared to baseline; whereas the FXS+autism group had shown no such activation. Despite these differences on the within-group analyses, there were no significant differences on the formal between-group analysis.

On the other hand, the finding in the cerebellum of a correlation between activation to neutral faces versus baseline and CSS scores in the FXS+autism group (Appendix 6) is perhaps supportive of the idea that autism is associated with activations in response to neutral stimuli. Although, given the small numbers included and the post hoc nature of the analysis, this should be cautiously considered. It is interesting, however, that the findings for the role of the cerebellum in social processing in autism have generally been that cerebellar activation is diminished in individuals with autism compared to typically-developing controls (Critchley et al., 2000; Deeley et al., 2007; Grezes, Wicker, Berthoz, & de Gelder, 2009; Herrington et al., 2007). However, in their meta-analysis of 350 fMRI studies examining the role of the cerebellum and social cognition, Van Overwalle *et al* suggest that cerebellar activity may actually increase when the level of abstraction in the task increases, and with it the demand on executive resource (Van Overwalle, Baetens, Marien, & Vandekerckhove, 2014). If neutral faces are considered to be more ambiguous and thus may appear more abstract than overtly emotional (in this case fearful) faces, then this may be a possible explanation for the finding. It is also of interest that increased activations to neutral faces, have also been reported in children (Thomas et al., 2001) and in patients with schizophrenia (J. Hall et al., 2008; Surguladze et al., 2006); alternatively suggesting that such a response may be associated with neurodevelopment more generally.

Response to fearful facial stimuli

In the contrast looking at activations to fearful faces > baseline, a cluster of significantly decreased activation was found in the left superior temporal gyrus in those with FXS+autism compared to those with FXS alone. This finding overlaps the previous findings in individuals with idiopathic autism (Di Martino et al., 2009), but also replicates the finding of Dalton of increased activity in the left STG in individuals with FXS compared to both typically-developing and autistic controls (Dalton et al., 2008). Interestingly, in the FXS group reported by Dalton, none of them had a clinical diagnosis of autism, and the group had relatively low average autistic traits as measured by the Social Communication Questionnaire (SCQ) (mean SCQ of 9). Thus, the FXS group studied by Dalton is likely to be comparable to our non-autism FXS group. Interestingly, as well as overlapping the region identified by Di Martino (2009) as having a higher likelihood of activation in controls compared to ASD, it also overlaps the more anterolateral cluster described by Philip (2012) as having a higher likelihood of activation in ASD subjects compared to control subjects.

In addition to the large significant cluster in the left STG, there was also a cluster at the level of a non-significant trend in the left cuneus. Whilst this is not a region generally discussed in either the fragile X or the autism literature as being of particular importance, the *right* cuneus has previously been identified as being hypoactive in fragile X on a sad faces > neutral faces contrast (Hagan et al., 2008). Unfortunately, the Hagan (2008) study does not provide details of

the relative levels of autistic symptomatology to allow further comment to be made.

Regression analyses

In the regression analyses, which examined correlations between a continuous measure of autistic features (the ADOS calibrated severity scale) and activations, no results were found on either of the main contrasts of neutral faces or fearful faces compared to baseline. However, the results in Appendix 6, examining the response to all faces versus baseline showed a cluster in the left cerebellum that contrasted with the ADOS CSS. In exploring the neutral faces and fearful faces baseline contrasts with a small volume correction based on the cluster found in the all faces versus baseline, a small cluster of significant correlation was found on the neutral faces contrast.

In the FXS literature, increased activation in the left cerebellum has previously been reported in a FXS group compared to controls on an auditory temporal recognition task (S. S. Hall et al., 2009) with both hypo- and hyper-activation reported in FXS subjects in a paradigm examining response to direct gaze (Garrett et al., 2004). Interestingly, however, left cerebellar activation has also been previously positively correlated with FMRP levels (Menon et al., 2004; Rivera et al., 2002) and mental age (S. S. Hall et al., 2009).

Further, in the broader autism literature, the cerebellum, and the left cerebellum in particular, has largely been associated with decreased activations in groups of autistic participants in a variety of domains including: basic social tasks (Critchley et al., 2000; Deeley et al., 2007; Herrington et al., 2007); complex social tasks (Silani et al., 2008; A. T. Wang, Lee, Sigman, & Dapretto, 2007) executive function (E. Daly et al., 2014) and attention (Keehn, Nair, Lincoln, Townsend, & Muller, 2016; Rahko et al., 2016). Motor tasks, in which the cerebellum plays a key (if not precisely defined) role in modulating (Manto et al., 2012); have also been associated with differential activations in autistic participants; with both hypo- and hyper- activations being reported (Allen & Courchesne, 2003; Allen, Müller, & Courchesne, 2004; Muller, Kleinhans, Kemmotsu, Pierce, & Courchesne, 2003). How the finding in the present study fits in the context of these previous studies is not clear, however, it raises interesting questions about the interaction. Of particular interest is that the finding appears to be being driven by response to neutral faces as noted earlier; the same finding as seen in the SEN group discussed in the previous chapter. Whilst the effect was statistically significant, given the small group size and the post hoc nature of this analysis, the possibility that this result may be a type I error cannot be excluded.

Effect of group (FXS vs. SEN)

In this analysis, the question is whether there is anything in individuals with FXS that differs in the processing of facial stimuli that is not accounted for by IQ alone. In the fearful faces > baseline contrast, the FXS group showed a significant cluster of greater activation in the left supplementary motor area (SMA) and a parallel cluster at the level of a non-significant trend in the right SMA. The SMA is known to play a role in emotion processing (Tomasino & Gremese, 2016) and it is possible that the increased activation in the fragile X cohort reflects differences in this regard. Indeed, as one of the regions originally reported as a 'mirror-neuron' area (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010), it is possible that this activation represents differential mirror-neuron activity in response to the stimuli. In their study of individuals with FXS, and their startle response to fearful and happy faces using orbicularis oculi electromyogram (EMG), Ballinger (2014) found significantly reduced response to fearful, but not happy, faces in individuals with FXS, compared to a typically-developing group. They also reported that,

"The group with FXS showed stronger potentiation than the group with DD, though this difference did not reach significance".

It is possible that this result reported by Ballinger reflects the same observation reported here; greater response to fearful, or perhaps just highly emotionally salient faces in FXS compared to individuals with idiopathic developmental delay. It is, of course, possible that instead, this finding reflects motor differences between the groups. However, by using the number of trigger activations as a covariate in these analyses, it is hoped that any motor effect

explaining the result will have been diminished. That no difference was seen in the primary motor cortex also supports the idea that this was not a more general motor difference.

The analysis considering the group by autism interaction revealed a significant cluster in the left superior temporal gyrus. However, when extracting the Eigenvalues to explore the nature of this interaction, it is clear that the result is driven by the earlier finding of differential activation in this cluster in the FXS individuals, with the difference between the two SEN groups (autism and non-autism) not being significant. However, in the previous chapter, a similar, albeit slightly more posterior, finding had been reported in the SEN cohort, i.e. that the autism group showed relative hypoactivation in the left supramarginal gyrus compared to the FXS non-autism group. Additionally, that the finding reported here in individuals with FXS overlaps with the previous findings of Dalton (2008) in FXS and Di Martino (2009) in idiopathic autism gives a degree of confidence that the finding reflects differences in emotion-processing that may be seen in autism, regardless of aetiology.

Conclusions

These analyses have shown that autism in individuals with fragile X syndrome is associated with a similar reduction in activation in the left superior temporal gyrus as is seen in individuals with both idiopathic autism compared to typically-developing controls (Di Martino et al., 2009) and in idiopathic autism compared to non-autistic individuals with FXS (Dalton et al., 2008). This supports the idea that autism in FXS may, at least in part, represent a good model for autism more broadly.

The finding of left cerebellar activation being correlated with degree of autistic features on a contrast examining response to neutral faces is an interesting finding, although was only found *post hoc*, in exploring the finding in the primary faces>baseline contrast. Whilst not reflecting findings in the broader autism or FXS literature, it nonetheless raises interesting questions about the possible nature of response to non-emotional, neutral stimuli, which have been of less interest previously than studies of more highly emotional stimuli. Further studies are required to clarify the nature of this relationship; it would be of particular interest to use an alternate baseline stimulus against which to compare the response to neutral faces; e.g. scrambled faces or, tonally-matched geometric shapes.

The analyses including both cohorts, produced an interesting finding of increased activation in the FXS group in bilateral supplementary motor areas in

response to fearful faces. The significance of this is not wholly clear, although raises the interesting possibilities of increased mirror neuron activity in fragile X or more broadly, increased responsivity to fearful facial stimuli, as previously suggested by Ballinger (2014).

Taken all together, it appears that in individuals with FXS, the presence of autism is associated with relative hypoactivation in left superior temporal structures, an observation that overlaps previous findings in idiopathic autism research. That other findings of differential activation reported in the broader autism literature were not found in the FXS cohort suggests that FXS appears to only partially share the same neural underpinnings as idiopathic autism.

This chapter forms the basis of the published paper included in Appendix 4 (McKechanie, Campbell, et al., 2019).

Chapter 7: Concluding remarks

Introduction

In attempting to address the relative paucity of functional imaging research in individuals of lower cognitive ability, this thesis has reviewed functional imaging in individuals with special educational needs (SEN) and also in individuals with fragile X syndrome, with particular reference to the impact of autism in these groups. The experimental chapters of the thesis then describe the investigation of the role of autism as a mediator of neural response to emotional stimuli in both individuals with special educational needs and fragile X syndrome. This chapter now provides an overview of the results from each of the chapters and attempts to synthesise these. Strengths and limitations of the work, and future research questions are discussed.

Summary of study findings

In the reviews of previous imaging findings, this study focused on both imaging in autistic individuals of low average cognitive ability, as well as individuals with fragile X syndrome, with varying degrees of autistic traits. In the review of imaging in autistic individuals with low average cognitive ability, the clearest finding was of generally lower activations in individuals with autism/ASD compared to the control groups of interest, across almost all contrasts considered. In the studies that considered connectivity (both effective and functional), these were less clear in their overall findings (finding both over- and under-connectivity); a result that is in keeping with reviews in the general autism imaging literature (Hull, 2017).

One issue that was clear, however, was that of the IQ of individuals in both main groups of interest and comparison group choice. On an individual level, each study had its reasons for its choice of groups. The reasoning behind this is explained to greater, or lesser, degrees in each of the studies; some with clearer reasoning than others. However, the cumulative effect is that the research appears to be very significantly skewed. Of the 54 papers reviewed, only one included a main group of interest with an average IQ <70. Further, in the 7 studies reviewed with main groups of IQ of below 85, none of them included a comparison group with an IQ in the low average (IQ 80-89) or borderline (IQ 70-79) range, although Reiter *et al* (2018) did include an average IQ typically-developing group as well as a high IQ typically-developing group to at least start to explore this area. As with any endeavour that involves individual choice, and in this case the exercise of academic choice by researchers, it is hard to argue that each study does not have its merits, and that it should not have been done. However, the cumulative effect is that the research evidence to date is not wholly representative of the population of all autistic individuals (or at least if it is instead generalisable, there is little evidence on which to base such an assertion).

In the review of functional imaging in fragile X syndrome, there was much greater heterogeneity of both the paradigms considered and the results found. However, a number of themes emerged. Firstly, as with the autism imaging studies, there was a general trend towards greater activations being seen in the

control groups than in the FXS groups. However, this appeared to be less strong a finding than it had been in the autism literature. Secondly, a number of the studies had correlated activation findings with FMRP and/or FMR1 expression levels. As might be expected, more typical levels of FMRP/FMR1 were generally associated with more typical patterns of activations. Finally, in the studies that examined responses in graded tasks (Klabunde et al., 2015; Kwon et al., 2001), differences between the groups became apparent at more complex levels of the tasks. Further, these differences were not always accompanied by difference in performance of the task.

In the imaging study of autism in the SEN group, there were two main findings; one expected, and one somewhat unexpected. Firstly, the non-autistic group showed clusters of significantly greater activation in the left angular gyrus/supramarginal gyrus and the left dorsomedial prefrontal cortex compared to the autistic group on the fearful faces > baseline contrast. In particular, the finding in the temporo-parietal junction overlaps previous findings and is close to significant clusters found in two previous meta-analyses of autism imaging (Di Martino et al., 2009; R. C. Philip et al., 2012) adding weight to the validity of the finding. The unexpected finding was that the autistic group showed increased activations to neutral facial stimuli. The reasons for this are not clear, however, a possible explanation may be that the relative ambiguity of the neutral stimuli made for complexity in interpreting them, which itself may have

been associated with excess neural activity. This is an area that requires more exploration.

In the fragile X syndrome imaging study, the within-group analyses of neutral faces > baseline contrast showed that the non-autism group had a cluster of significant activation in bilateral posterior structures, whereas the autism group had no significant clusters. On the contrast of fearful faces to baseline, both autism and non-autism groups had significant clusters in bilateral cuneus and calcarine; albeit that the cluster was of greater extent in the non-autism group. Both groups also had further, smaller clusters of significant activation to this contrast: the non-autism group in the left ventral striatum and left precentral gyrus, and the autism group in the left supplementary motor area. In the between-group analyses; there was one clear finding of significantly reduced activation in the FXS+autism group compared to the FXS non-autism group in a cluster in the left superior temporal gyrus during the viewing of fearful faces versus baseline. This also overlapped the cluster reported in an autism meta-analysis by Di Martino (2009), as well as the cluster reported in a previous FXS imaging study by Dalton (2008) and suggests, albeit tentatively, that autism in FXS may have similar underpinnings to idiopathic autism.

When considering the neural response to fearful faces, both imaging studies showed clusters of significantly different activation between the autistic sub-group and the non-autistic subgroup in each study. Whilst the clusters in the

left superior temporal gyrus in the SEN imaging study and in the FXS imaging study do not overlap, given that both clusters have been associated with hypoactivation in autistic individuals on social tasks (e.g. Di Martino (2009), Herrington (2007), Kennedy & Courchesne (2008)) there is reason to believe that they may be tapping the same underlying process. This is consistent with the idea that there are findings which may be robust within autistic individuals both across groups of differing cognitive ability; but also between idiopathic and genetically-defined groups. However, it is clear that further research is required to confirm this before that can be assumed. In particular, the inclusion of typically-developing and cognitively more able autistic participant groups, would help to further explore the nature of these relationships.

Summary review of the experimental aims and hypotheses

In this series of experiments, it was firstly demonstrated that it was feasible to successfully conduct functional imaging in individuals with varying degrees of intellectual impairment; individuals who, as shown in chapters 2 & 3, are typically excluded from studies. Secondly, the results showed responses in the BOLD signal commensurate with the extant literature, demonstrating that the method paralleled prior work in those of greater cognitive ability. Thirdly, and finally, the results showed relative hypoactivation to fearful faces in autistic subgroups of the two separate samples. In addition, results relating to relative hyperactivation in the autistic groups to neutral faces were found. This was an

unexpected, although interesting finding, the potential importance of which has been discussed, although it is possible that the choice of neutral facial stimuli may have been a factor that influenced these results. Further investigation incorporating out-of-scanner tasks may help to clarify the answer to this question.

Reflections on methodology

This research occurred in the context of a research department that had undertaken considerable previous brain imaging in individuals with major mental illness. There had also been structural imaging in individuals with special educational needs, as well as structural and functional imaging in autistic individuals with typical or enhanced cognitive ability. However, functional imaging in more severely affected individuals was novel. Building on the previous experiences of imaging, it was possible to develop a protocol that appeared acceptable to participants, and when considering those who dropped out during the desensitisation process, it is not clear that further adaptations could have facilitated a scan for them, at least not without undue pressure being brought to bear. Of all the steps in the process, the presence of the mock scanners was undoubtedly the most valuable; both of them allowing for a different experience. The first mock scanner was small and deliberately designed not to be intimidating. It allowed for a low-key introduction to the modality for participants and families. In contrast, the mock scanner housed at the imaging centre was very realistic and, barring the lack of the physical

vibrations, provided in many ways a very realistic experience. In terms of desensitisation, this step felt important for at least some of the participants in better preparing them for the real scan. Unfortunately, shortly following the conclusion of the imaging study, this mock scanner was sold in order to create further space for another research MRI scanner. Only time will tell as to whether its absence makes participation more challenging. It is anticipated that extended desensitisation on the first mock scanner, or perhaps (very expensive) desensitisation using the real scanner may be necessary to best prepare participants for their scan.

Whilst the focus of this thesis was on functional imaging, the structural scans simultaneously acquired to allow the functional imaging analysis could themselves be analysed either alone, or in tandem with the functional scans. Indeed, the study by Hall et al (2013) incorporated both functional and structural analyses, the results of which showed grey matter tissue loss corresponding with a functional connectivity finding. Whilst it had been hoped to include resting-state functional connectivity as part of the imaging protocol, and indeed scans were acquired from a small number of participants, it was evident that a third scan was, for many participants, too much to undertake at one visit. Thus, it was decided to stop routinely collecting these scans. However, it is hoped that having shown that scans in such individuals are possible, and hoping that for most of the participants the experience was a positive one, that future functional connectivity scans will be possible.

Nonetheless, the results from the connectivity scans that were acquired are currently being analysed in tandem with the preclinical imaging results to help shine a light on what parallels may, or may not, exist across species with FMR1 gene changes.

In addition to the analyses that were considered in this thesis, there are of course other ways in which the data could have been considered. For example, subscales of the ADOS, or other measures of autistic traits, could have been assessed against the findings. Further, it would have been possible to undertake out-of-scanner assessments of the individuals understanding and response to faces / emotional faces. This may have helped to shine a light on the imaging findings. In particular, it is not clear the degree to which the differential responses to the faces represents differential understanding of the facial emotion. Whilst that was not the specific question being considered here, exploration out of the scanner of individuals' understanding and interpretation of the facial affect would help to interpret the results.

Implications for practice

The principal implication of this study is that imaging is possible in individuals with significant degrees of intellectual disability with the use of mock scanning environments. Thus, clinicians should not automatically exclude the possibility of acquiring a brain scan when considering investigations. In some cases, such investigations are put off until such time as they become urgently necessary

(and thus can justify sedation or anaesthetic); whereas with the use of suitable preparation and desensitisation it may be possible to acquire imaging that is helpful to the clinician and not distressing to the individual. Whilst use of such methods is a more routine practice in paediatrics, it is less common in adult radiology. As noted earlier, availability of suitable mock scanning environments can be difficult and thus consideration should be given to how best to prepare individuals for scans. In some cases that may be through visits to the radiology department, possibly facilitated by learning disability liaison nurses where they exist or by use of materials prepared by the hospital or the MRI manufacturer.

Beyond this very practical implication, there is the more general point that consideration should be given in future studies to potential selection bias in recruitment for imaging studies. Whilst clearly not all studies can include all participants; there is nonetheless a place for at least acknowledging this as a possible limitation of studies. Further, that this study has demonstrated the possibility of including individuals more intellectually impaired than is typical for imaging studies, and how the results at least partially replicate findings in previous studies; should encourage the inclusion of a broader spectrum of participants in future study.

As discussed in chapter 1, it has long been recognised that fragile X syndrome is associated with autism; with autistic traits being more common, and a formal diagnosis of autism being appropriate in approximately 30% of individuals.

However, the exact nature of the relationship between the two conditions has been less clear. In the review of prior functional imaging studies in fragile X syndrome, the presence of autistic traits or diagnoses had not particularly been used as a way to explore the data, although comparisons between individuals with FXS and individuals with ASD had been made. Where this study perhaps adds to the literature is in demonstrating that individuals with FXS who have high levels of autistic traits when compared to individuals with FXS alone show differences in brain function analogous to those seen in individuals with ASD compared to neurotypical participants. This can give a degree of confidence that the making of a diagnosis of ASD alongside one of FXS may have validity and, hopefully for the individual and their family, some utility.

Whereas it has long been recognised that autistic individuals can have a broad range of cognitive ability, it has been far from clear that the results of prior neuroimaging studies were of relevance to those of lower cognitive ability. Whilst the studies in this thesis cannot provide a definitive answer to this, they hopefully go some way towards extending the relevance of prior works, as well as being a novel contribution to the literature in and of themselves.

Potential methodological limitations

As with any study, there are a number of methodological points which may limit the generalisability of the study findings, and thus merit remark. Firstly, in both the SEN and FXS studies, the participants may not be representative of the

population they are drawn from. For the SEN cohort, the inclusion criteria were very broadly defined in the parent study, with the end-phenotype of teacher-estimated special educational need encompassing a broad spectrum of individuals. Whilst in the most part participants fitted the guideline estimated IQ of 50-80, this was not the case for all participants, with some having IQs outwith this range. Nonetheless, the use of this educational phenotype takes into account a wider, and potentially more valuable set of information about individuals than a simple measure of IQ alone. Further, the individuals who participated in this imaging study may themselves not be representative of all of those with a special educational need; with some factors making participation in a study less likely for some individuals.

For the FXS sample, the participants almost certainly were not wholly representative of the broader FXS population. Whilst they were likely to be more representative than many of the studies that have gone before (by virtue of including more males and those of a lower IQ), a number of potential participants who originally expressed interest about the study, did not go on to complete the study by virtue of a number of barriers to participation (e.g. travel, staying still, scanner noise). Further, there were almost certainly individuals and their families who discounted participation at an even earlier stage and did not even make contact. It is likely that those who chose not to participate, or who did not progress to completing a functional scan, were more severely affected. In a similar vein, the low use of medication in the sample studied likely

represents both prescribing practices in the U.K., but also perhaps a degree of selection bias; with those more severely affected (and thus more likely to be on medication) less likely/able to participate. So, whilst the study deliberately tried to include as representative sample as possible of those with FXS, it was still likely not fully representative.

Whilst both studies had sample sizes that compare reasonably well with similar studies (as reviewed in chapters 2 and 3), a larger sample size is required before any definitive conclusions can be drawn. In particular, the regression analyses in the autism-only groups would have benefited from the increased power that larger numbers would have afforded.

In this study, the impact of autism was considered *within* groups of individuals; one group with special educational needs, and the other with fragile X syndrome. Given that both of these groups differ from the general population (and indeed are defined by their difference (either educational or genetic)) the focus was not on if, and how, the groups differed from the general population. However, by not having a typically developing comparison group in either study, the results and conclusions are potentially limited by not being able to make wider comment on how these groups differed from the general population. In terms of comparison or control groups, previous studies have included a variety of comparison groups: typically-developing controls, ASD controls, typically-developing developmental age-matched controls or

developmental delay controls all having been used. It would, of course, have been interesting to include further comparison groups; however, that was beyond the scope of this study. In particular, as noted earlier, the inclusion of a typically-developing group and an autistic group of average cognitive ability would help to explore the nature of the relationship further, rather than relying on previously-reported works.

The use of the ADOS as a measure of autistic traits has its limitations. Most importantly is that the measure itself is an observational one and is reliant on what occurs during the interview. Various factors may influence the interview and mean that it is not wholly representative of the individual. Whilst the interview and the 'presses' therein are standardised to reduce the chance of this, it is nonetheless not a perfect tool. Further, there is limited work on the validity of the ADOS in intellectually disabled populations, and in particular the Module 4 for adults with an intellectual disability. This said, the bimodal distribution of both ADOS total and CSS scores gives a degree of confidence that our groups represented individuals with significant differences in social communication and interaction. Indeed, had the lower threshold of $\geqslant 7$ for autism spectrum disorder been used as published in the ADOS, the groups would have remained unchanged.

During the acquisition of the imaging data, a trigger was used for the participants to indicate when they had seen a face, with participants needing to

respond successfully on more than 80% of faces to be included in further analysis. However, the scanning facility unfortunately did not have in-scanner eye-tracking available, and as such it can not be known for how long, or with what pattern, the participants visually attended to the stimuli. Given that both FXS and autism are associated with differences in gaze patterns, and that there may be group differences on gaze, it is not possible to be confident that the results do not represent differences in gaze, either instead of, or as well as, differences in underlying neural processing. It is hoped that future research will be able to incorporate eye-tracking hardware.

Direction of future research

Whilst this thesis has presented the main analyses considered to be of interest, there remain a number of other analyses that could be conducted on the data collected. When considering autistic symptomatology, this thesis has primarily concerned itself with considering groups of those considered to be autistic versus those who were not. However, it would be possible to analyse the data through a different lens, and instead consider different domains within the autistic phenotype (e.g. restricted and repetitive behaviours or particular aspects of social communication) and examine their relationship with functional MRI responses. This may help to elucidate whether particular domains or symptoms may be of particular relevance for different groups of individuals. Another obvious way to consider the data would be to correlate results with measures of cognitive ability, either with a relatively crude measure such as IQ, or looking at particular domains of cognition more specifically.

In both the SEN and FXS cohorts, there were results that suggested an increased response in the autistic groups to neutral stimuli. It would be of interest to pair future imaging paradigms with out-of-scanner testing to probe further the nature of this relationship. In particular, it was suggested in the thesis that ambiguity in perceiving the neutral faces may be contributing to the results seen; this could be probed further in future studies to consider whether, and to what degree, it is a factor. For example, by considering the accuracy and speed with which individuals can identify the emotion represented in each

image, it would be possible to correlate these against their fMRI activations and understand to what degree this is a factor in the results seen in this study.

Whilst collecting the functional imaging data in the FXS imaging study, a number of resting-state functional MRI scans were also collected. Collection of further rs-fMRI scans continues and will be the subject of future work. This modality is of particular interest as a potential translational tool and in parallel with this ongoing work, fundamental science colleagues are conducting similar scans in existing rodent models of FXS. In this collaboration, parallel patterns of connectivity between the human and preclinical models are emerging. A poster of the preliminary results is included at Appendix 7 (Smith et al., 2019) and, now that the methodology to scan individuals with FXS has been established, this is an area that can be developed going forward. As well as looking at baseline levels of functional connectivity, there is also the possibility of examining whether potential treatments impact functional imaging (both resting state and task-based fMRI) differences in both laboratory models and affected people, and thus potentially allow for the screening of medication ahead of any larger human clinical trials.

A major goal of this programme of study was to establish whether it was possible to extend functional imaging studies to those who are more intellectually impaired, and who have traditionally been excluded from study. Having refined methods for scanning individuals with intellectual disability and

demonstrated that it is possible to investigate this population with fMRI methods, there is now the possibility to use these methods to answer further questions. Whilst overlaps in social brain function were found in this study, it is not known if these extend to other cognitive domains. The use of other imaging paradigms (many of which were reviewed in chapters 2 and 3) would allow examination of a number of possible questions. Beyond fragile X syndrome, it would be of interest to use the methodology as applied to other participant groups; in particular other monogenic forms of intellectual disability, to examine whether patterns of activation or connectivity span diagnostic groups. In those who are even more intellectually affected than those studied here, the use of functional MRI which does not need participants to respond in any way (e.g. resting-state MRI) is of particular appeal.

The studies described in this thesis focused on functional imaging, although structural imaging data was also acquired. This was used for the pre-processing of the functional imaging data, although could be used itself to gain further insights into the nature of autism and fragile X syndrome. In particular, it could be powerful to examine whether any of the functional imaging results reported here, have structural imaging correlates; perhaps in a similar way as reported by Hall et al (2013).

Whilst in the fragile X study, participants were considered as a genetically-homogenous group (and indeed fragile X syndrome is defined by the presence

of a particular genetic change); this inevitably exists in the context of each individuals' genetic background, and in future research it would be of interest to start to consider polygenic background factors in the analyses. Although much larger samples would be necessary to allow this, considering the interaction of polygenic risk scores in analyses could potentially be powerful. It is possible that there may be factors here that can be elucidated that help explain some of the results found here. Of particular interest would be considering whether polygenic risk scores in fragile X syndrome (or indeed other monogenic forms of ID of interest) are associated with the extent or nature of autistic traits or imaging findings. That FMRP is an mRNA binding protein that interacts with >1000 other proteins, means that it has potentially wide-ranging impact and it may be that polygenic factors in the interactors of FMRP may be of importance.

Conclusions

The autism literature and fragile X syndrome functional imaging literature has to date concentrated on individuals not necessarily representative of the whole spectrum of those affected. In the autism literature, this has been by studying those of average or enhanced intellectual ability, and in the FXS literature there has been a relative over-representation of females and those less intellectually impaired.

One of the potential problems of this is that if our understanding of the condition is based on a skewed sample, then we should not be surprised if treatments developed on the back of such evidence do not prove efficacious when used to treat all. Further, FXS is often touted as a model for autism more broadly and it would appear that the development pipelines for drug trials in FXS consider possible future use in autism more broadly.

While this thesis, and the imaging studies herein do not solve this issue, it is hoped that they may go some way towards helping. Firstly, by providing some evidence that there are parallel patterns of response that generalise between the extant literature in ASD and FXS and the more severely affected individuals studied here. But also by perhaps serving to shine a light on the issue and suggest methods by which more severely affected individuals can be included.

Finally, the results of the imaging studies *do* suggest that autism, whether idiopathic or associated with FXS, may be associated with partially, if not wholly, similar effects on the neurological underpinnings of facial emotion processing.

Appendices

Appendix 1: McKechanie *et al,* The Lancet Psychiatry (2020)

The Never-Changing Face of Fragile X?

The Lancet Psychiatry 2020
7 (5), e26

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The never-changing face of fragile X?

Dr William W Ireland (1832–1909) was medical superintendent of the Scottish National Institution for the Education of Imbecile Children at Larbert, Scotland, in the 1870s, and published extensively on matters psychiatric, neurological, and historical.[1,2] In his 1877 book, *On idiocy and imbecility*,[3] which came to be considered the definitive work on the topic, he attempted to categorise the causes of individuals who would now be recognised as having an intellectual disability. In the chapter on so-called genetous idiocy, he noted that, "The most common accompaniment of genetous idiocy, is what has been variously called the keel-shaped, or saddle-shaped, or vaulted palate." Accompanying this description is a sketch (figure) of an individual with a vaulted palate, and whose face we believe bears a striking resemblance to many individuals with fragile X syndrome. Such prima facie evidence is supported by the fact that arched palates are a common feature in fragile X syndrome,[4] which is the most common cause of inherited (or genetous) intellectual disability.

Using this sketch and the scant clinical information available, we used the Face2Gene clinic application[5] to assess the individual. On facial features alone, the application returned mucolipidosis type IV, fragile X syndrome, and Smith-Lemli-Opitz syndrome as the top three suggested syndromes. Including the known clinical information of high palate, and our further observations of large forehead and apparent poor eye contact, the application returned fragile X syndrome as the most probable diagnosis.

Figure: **Sketch appearing in W W Ireland's 1877 book**

Although not conclusive, we suggest that this figure, which appeared in print more than 140 years ago, might be one of the earliest depictions of an individual with fragile X syndrome. The case also highlights the potential value of facial phenotype-to-genotype software in support of clinical diagnostics and shows the possible application of such software to artworks more broadly.

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Appendix 2: McKechanie *et al*, British Journal of General Practice (2019)

Fragile X-associated conditions: implications for the whole family

British Journal of General Practice 2019

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Fragile X-associated conditions:

implications for the whole family

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INTRODUCTION

Fragile X syndrome (FXS) is a triplet-repeat expansion disorder of the X chromosome, with repeats of more than 200 (sometimes referred to as the full mutation) causing FXS and ~59–200 repeats (the so-called premutation) being responsible for a variety of clinical presentations. Clinicians in primary care should be aware of these conditions and in particular be vigilant for common comorbidities to allow for early treatment. This article summarises the common issues for individuals with FXS and carriers of the premutation.

HOW ARE INDIVIDUALS WITH FRAGILE X SYNDROME TYPICALLY AFFECTED?

FXS is the most common inherited cause of intellectual disability, occurring in approximately 1 in 3000–4000 males and 1 in 6000–8000 females. Although the genetic underpinnings of FXS are similar across individuals, the manifestations vary widely and in some ways there is no 'typical' presentation. Nonetheless, males with the syndrome generally have an intellectual disability ranging from mild to severe, whereas females are much more variably affected (due to random X-inactivation) and can range from being essentially asymptomatic to having a severe intellectual disability. There are a number of common physical comorbidities associated with the syndrome including epilepsy (~25%), mitral valve prolapse (≤80%), hyperextensible joints, and an increased risk of inguinal hernias. Anxiety, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorders (ASDs) are also significantly more common. Hyperarousal and sensory hypersensitivity are frequent symptoms, which may occur across a range of diagnoses. It is worth noting that, although one-third to two-thirds of individuals with FXS may meet criteria for an ASD, the presentation often varies subtly from that seen in idiopathic ASDs. In particular, some traits such as social difficulties and atypical eye contact may have very different underpinnings in FXS as compared with ASDs.[1]

WHAT INTERVENTIONS ARE RECOMMENDED IN FRAGILE X SYNDROME?

To date there is no medication specifically for core FXS symptoms (although this is an active area of clinical trial research). However, active vigilance in primary care is recommended for common comorbidities, which often impact quality of life most. Particularly, epilepsy, anxiety, and ADHD are common in individuals with FXS,[2] and active treatment for these should be considered. As ever, clinicians should be aware of the possibility of diagnostic overshadowing, meaning that treatable comorbidities may go untreated if attributed to core FXS symptomatology. The Royal College of General Practitioners' syndrome-specific medical health check guide for FXS provides further details on common comorbidities to be routinely reviewed in primary care.[3] Where necessary, referral to the local community learning disability team should be considered to support optimal diagnosis and treatment (Box 1). Multidisciplinary assessment is key, particularly occupational therapy for functional and sensory assessment, and speech and language therapy for communication support. Many families find the support of specific fragile X support organisations to be of help; the Fragile X Society (https://www.fragilex.org.uk/) being the UK-based organisation for this.

HOW ARE CARRIERS OF THE FMR1 PREMUTATION TYPICALLY AFFECTED?

The FMR1 premutation is carried by up to 1 in 150 females and 1 in 470 males. Historically, carriage of the FMR1 premutation (which is found in families with FXS) was not thought to be associated with any morbidity. However, more recently it has become apparent that it brings with it the increased likelihood of

Box 1. Genetic diagnosis in intellectual disability

- Seeking a genetic diagnosis for an intellectual disability is standard care in paediatric services, although there are large numbers (especially of adults) who have either never had genetic testing or for whom it was so long ago so as to be of limited meaning.
- It is estimated that routine genetic testing in individuals with intellectual disability can identify a genetic cause in up to 20%, with more detailed sequencing (not yet routinely available outside research studies) taking this up to over 50%.[4]
- Genetic testing should ideally be a routine part of care and should be considered where it has not been done previously. Clinicians should discuss with their local genetics department and community learning disability team to establish local practices.

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a number of conditions, notably including fragile X-associated premature ovarian insufficiency (FXPOI), fragile X-associated tremor and ataxia syndrome (FXTAS), and higher rates of mood and anxiety disorders.

Fragile X premature ovarian insufficiency (FXPOI) affects approximately 20% of female premutation carriers with increased infertility, and menopause occurring on average 5 years early.[5] A small proportion of women will experience the menopause at a much earlier stage, in their 20s or 30s. Notably, women with the full mutation do not, however, experience FXPOI.

Approximately 45% of males and ≤16% of females carrying an FMR1 premutation develop fragile X-associated tremor-ataxia syndrome (FXTAS). This neurodegenerative condition usually occurs over the age of 50 with risk of manifestation and severity increasing with age. Apart from the core symptoms of action tremor and/or ataxia (gait difficulties and disturbed limb coordination in particular), it may present with mild Parkinsonism, cognitive decline (short-term memory and executive function deficits), neuropathy, neuropathic pain, and autonomic dysfunction. Regarding the treatment of FXTAS, referral to neurology should be considered, where symptomatic treatments for action tremor, Parkinsonism, neuropathic pain, and mood/anxiety problems may have a role. One small trial of memantine for cognitive effects showed no effect, although there may be a role for cholinesterase inhibitors. As with the general population, treatment of contributing factors including hypothyroidism, vitamin B12 and folate deficiency, and cerebrovascular risk should be considered. Long-term care of FXTAS is complex and requires a multidisciplinary approach.

Studies of premutation carriers report a broad range of other medical problems,[6] commonly including thyroid problems and mood and anxiety disorders. These should be treated as they would be in the general population, acknowledging the additional stressors associated with providing care for someone with special needs. As is the case for anxiety and mood disorders in the general population, clinicians need to take particular care not to attribute valid concerns, for example, about a child, to symptoms of psychiatric disorder.

WHAT ARE THE IMPLICATIONS FOR FAMILY PLANNING?

Typically, the expansion from premutation to full mutation occurs during maternal meiosis; whereas female premutation carriers may experience expansion to a full mutation in their children, male carriers usually pass on the premutation unchanged. The birth of a child with FXS is often the first indication that the family carries the fragile X premutation, and thus there are implications for both the immediate and wider family.

For the mother with a child with FXS already, there will be a high chance that the premutation will expand again in future pregnancies; children inheriting the faulty gene (1 in 2) are likely to have full-mutation FXS. When members of the extended family are identified as carrying the premutation but who do not have children with FXS, the likelihood of expansion into the full mutation is more variable, depending on a number of factors including specific premutation repeat length in the premutation carrier (the risk of expansion is increased with increasing length). Similarly, for those with premutations identified in other screening programmes, the risk of the repeat expanding is more variable. Contact with genetic counselling will be helpful to consider all available options for family planning, which for some may include prenatal testing or preimplantation genetic diagnosis. This can quite understandably be a very stressful time for families and a cause of considerable strain.

Where an individual is identified as carrying the premutation through extended family screening, this brings its own specific issues and potential stresses, for example, not only the news that a woman may have an affected child but also the finding that ovarian reserve may be reduced because of FXPOI leading to difficulty in achieving a pregnancy.

CONCLUSION

Changes in the FMR1 gene are not only associated with both the fragile X syndrome, but also with a wide range of clinical manifestations in family members carrying the premutation. Clinicians are advised to be vigilant for common comorbidities, allowing early diagnosis, referral, and treatment. The familial nature of these conditions is of importance to general practice.

Provenance

Freely submitted; externally peer reviewed.

Competing interests

The authors have declared no competing interests.

Appendix 3: Table of functional imaging in autism review.

Table A1

All studies reviewed in ASD functional imaging review

Author (year)	Autism group					Control matching criteria	Task design
	N (M:F)	Mean age (sd)	Mean IQ (sd)	Diagnosis	Diagnostic measures		
Abbott (2018)	44:6	13.2 (2.7)	VIQ 102.8(17.1), PIQ 105.0 (17.1)	ASD	DSM-IV-TR, DSM-5, ADOS-2	Age and head motion (RMSD)	Eyes open, Functional connectivity
Alaerts (2016)	42:42	M 12.99 (3.05) F 13.3 (3.91	M 101.34 F 101.36	ASD	DSM-IV-TR, ADOS, SRS, ADI-R, used ABIDE dataset	Head motion (frame-wise displacement)	Eyes closed or open looking at crosshair.
Balsters (2016)	130:130	13.94 (4.3)	106.82 (12.86)	ASD	As per Di et al, used ABIDE dataset	Age, full scale IQ and head movement	Combination of sMRI, rs-fMRI and FC analyses
Barbeau (2015)	19:3	20.3 (5.5)	98.6 (10.7)	ASD	DSM-IV, ADI-R,	PIQ, Raven	Poffenberger task for

					ADOS-G	percentile and age	fMRI, functional connectiveity and DWI
Bernas (2018)	21:3	Set 1 15.5(1.0) Set 2 13.7 (1.2)	Set 1 116.7 (5.0) Set 2 VIQ 92.9 (18.8) PIQ 101.9 (14.7)	ASD	Multidisciplinary consensus diagnosis, ADOS, SRS and SCQ.	Age and IQ.	rs-fMRI
Brieber (2010)	15:0	16.42 (2.28)	108.53 (15.34)	ASD	ADOS-G, ADI-R, clinician review	Age and IQ.	Coherent motion-processing
Choi (2015)	27 (sex not broken down)	9.9 (2.5)	105.9 (14.7)	ASD	ADI-R-K, ADOS-K	Age and IQ.	Social reward task
Clery (2013)	11:1	28 (7)	114 (21)	AS	DSM-IV-TR, ADOS-G	Age	Visual oddball

Daly (2014)	14	31 (13)	115 (13)	Autism	ICD-10, ADI-R, ADOS	Gender, age, IQ	Go/No-Go task, pre- & post- tryptophan depletion
Daly (2012)	14	31 (13)	115 (13)	Autism	ICD-10, ADI-R, ADOS	Gender, age, IQ	Incidental processing of dis- gust, fearful, happy, and sad facial expressions.
Damarla (2010)	11:2	19 (5.5)	109.5 (8.7)	Autism	ADI-R, ADOS-G	age-, IQ-, and gender-matched	Embedded figures task fMRI, functional connectivity
Datko (2016)	8:1	13.1 (2.59)	96.1 (15.6)	Autism	ADOS	age	rs-fMRI
Falahpour (2016)	Study 1 67:9 Study 2	16.1 (4.9) 14.3 (2.4)	106.6 (18.1) 106.3 (18.0)	ASD	ADOS	age, sex, NVIQ, FIQ, PIQ, handedness, amount of	fcMRI

	28:4					motion, and eye status at scan	
Fan (2014)	24:0	18.4 (2.8)	107.0 (11.2)	ASD	DSM-IV, ADI-R	Matched (no detail)	Empathy-eliciting stimuli depicting physical bodily injuries
Gabrielsen (2018)	LVCP 14:3 HVCP 15:5	12.26 (3.34) 12.64 (2.87)	54.00 (17.50) 106.85 (13.64)	ASD	ADOS-2	Matched (no detail)	fcMRI watching Inscapes movie (Vaderwal 2015)
Gadgil (2013)	13:3	31.8 (12.0)	109.6 (12.1)	ASD (autism & AS)	ADOS-G, DSM-IV	age, gender, IQ and socioeconomic status	Block design, hierarchical shape-recogni- tion task

Gooskens (2018)	17:9	11.33 (1.07)	108.8 (16.67)	ASD	ADI-R	Age, gender	Stop-signal task
Groen (2010)	12:4	15.3 (1.6)	100.4 (20.6)	Autism	ADI-R	age, gender, handedness, and IQ	Sentence comprehension task
Guo (2016)	UCLA 17:1 Leuven 9:3	15 (1.8) 13.7 (1.2)	103.1 (13.1) 96.3 (13.8)	Autism	ADI-R, ADOS	Age	rs-fMRI functional connectivity analysis
Haigh (2016)	12:3	26	88-131 (only range given)	Autism	DSM-IV, ADOS-G, ADI	Matched (no detail)	one-back letter detection task presented at fixation (to control attention) while task-irrelevant sensory stimulation was de-livered to the different modalities

Hames (2016)	8:3	20-28 (only range given)	Not stated	ASD	Various, mainly via state approval for ASD services	No detail	Visual, Auditory, and Cross Modal Sensory Processing tasks
Hames (2016)	4:2	17.33	"High-functioning"	HFA	ADOS	No detail	Child Attentional Networks Task during fMRI
Hesling (2010)	8:0	23.38 (2.10)	VIQ 89 (7.89)	HFA	DSM-IV, ADI-R	age	Passive listening to speech stimuli, eyes closed
Holt (2014)	33:16	M 14.66 (1.6) F 14.45 (1.95)	M 108.7 (16.3) F 98.13 (11.05)	HFA, AS	ADOS-G, ADI-R	Age	Reading the mind in the eyes task fMRI

Itahashi (2014)	39:7	31.11 (8.14)	105.8 (14.12)	ASC	DSM-IV	Age, gender	rs-fMRI
Karten (2015)	10:2	12.4 (3.8)	Not reported, “with language disability”	ASD	DSM-IV, ADI-R	Age, handedness	Passive listening task, BOLD fMRI and functional connectivity analyses.
Keehn (2016)	14:2	14.2 (1)	VIQ 112 (17) PIQ 112 (14)	ASD	DSM-IV, ADI-R, ADOS	age-, nonverbal IQ-, and motion	Serial visual presentation paradigm
Keehn (2013)	19:0	13.83 (2.75)	112.9 (11.8)	ASD	DSM-IV, ADI-R, ADOS	age-, IQ-, gender-, handedness-, and motion	Visual search paradigm, fc-fMRI
Kestemont (2016)	9:3	30.17	No comorbid ID	ASD	Clinical Diagnosis from psychiatrist or	sex	Causal attribution task

					psychologist		
Kim (2015)	16:1	10.89 (2.06)	112.67 (12.73)	ASD	ADI-R-K, ADOS-K	Age, IQ	Happy, fearful, neutral faces task, fMRI,
Kleinhans (2016) (secondary analysis of Kleinhans 2011 data)	25:2	23.57 (6.60)	110.81 (15.68)	ASD	DSM-IV, ADI-R, ADOS	Age, gender, verbal IQ, performance IQ, or full-scale IQ	Habituation to fearful faces, houses, scrambled images.
Kleinhans (2011)	28 total	23.57 (6.60)	113.3 (14.22)	11 ASD, 15 AS, 2 PDD-NOS	DSM-IV, ADI-R, ADOS	age, IQ, and behavioral performance.	Passive fMRI block-design; fearful faces, houses, scrambled images.
Krach (2015)	16:0	21.5 (2.9)	VIQ 117.5 (14.4)	ASD (1 autism, 14 AS, 1 atypical autism)	DSM-IV, ICD-10, ADI-R, ADOS	Sex, age, VIQ	Images of physical and social pain/neutral conditions, participants asked to evaluate.

Lo (2013)	20:0	13.7 (2.8)	101.3 (13.8)	Autism	DSM-IV, ICD-10, ADI-R (Chinese version)	age, gender, and handed-ness	Semantic meaning judgement task, discriminating maning -related and -unrelated pairs
Lu (2015)	19:4	11.98 (3.42)	93.44 (5.35)	ASD	ADI-R, ADOS	Age	Faces of Self vs other; analysis of BOLD signal in cingulate cortex
Masten (2011)	18:0	14.0 (2.4)	105.0 (9.5)	ASD	ADOS, ADI, DSM-IV	Age, sex	Cyberball, fMRI; investigation of peer rejection
Maximo (2013)	25:4	13.8 (2.4)	107.9 (19.0)	ASD	ADI-R, ADOS	Age, handedness, non-verbal IQ, and motion	rs-fMRI
Morita (2012)	14:1	23.7 (4.3)	105.4 (11.7)	ASD	DSM-IV-TR,	Age, sex, IQ	Faces of Self vs other;

					DISCO		BOLD analysis
Mueller (2013)	9:3	35.5 (11.4)	111.3 (13.4)	HFA	ICD-10	Age, sex	rs-fMRI
Nijhof (2018)	15:11	32.8 (8.4)	106.4 (16.0)	ASD	Clinical Dx + ADOS	Gender	fMRI of explicit and spontaneous mentalizing.
Oberwelland (2017)	16:0	14.2 (3.52)	111 (17.4) – from 15 participants	ASD	ADOS-G, ADI-R	Age, IQ	Joint attention task, either initiating or responding to gaze for JA
Ohta (2012)	21:3	30.2 (7.6)	108.7 (12.7)	ASC (12- AS, 9 HFA, 3 PDD-NOS)	DSM-IV, DISCO	Age, sex	Visual target detection task under high or low perceptual load
Pereira (2018)	18:4	17.45 (3.29)	99.77 (9.5)	ASD	DSM-5, ADI-R	Age, IQ	Resting state - functional connectivity
Rahko (2012)	17:8	14.8 (1.6)	106.4 (17.53)	19 AS, 6	ADI-R, ADOS,	Age, sex	Block-design happy &

			only for 22 of 25	HFA	DSM-IV		fearful faces paradigm
Rahko (2016)	20:8	14.6	94.4	20 AS, 8 HFA	ADI-R, ADOS, DSM-IV	Age, sex	visuospatial n-back task
Rausch (2016)	19:1	16.23 (3.18)	102.3 (13.57)	Autistic Disorder	DSM-IV, ADI-R	age, sex, and handedness and verbal, performance, and full-scale IQ	rs-fMRI
Schulte-Rüther (2011)	18:0	27.4 (9.3)	106.6 (10.5)	Y HFA, 7 AS	DSM-IV, ICD-10	Age, sex, IQ	Emotional faces paradigm, task to either empathize with person, or think about how participant felt themselves seeing the picture

Shukla (2010)	25:1	13.7 (0.6)	VIQ 109.6 (3.1) PIQ 112.1 (2.8)	ASD	ADI-R, ADOS,	Age, FSIQ	Regional homogeneity-functional connectivity MRI (ReHo) during CPT (visual search)
Starck (2013)	18:6	14.9 (1.4)	107.3 (16.9)	17 AS, 7 Autism	ADI-R, ADOS, DSM-IV	Age, sex	rs-fMRI, eyes open, fixation cross
Tam (2017)	19:3	34.1 (11.5)	106.7 (14.0)	ASD	ADOS	Age, sex	Emotional faces n-back task
Utzerath (2018)	19:3	12-18	> 85	ASD	DSM-5, ADI-R	Age, sex, IQ	Perceptual expectations paradigm
Vaidya (2011)	11:4	10.78 (1.29)	113.85 (15.4)	ASD (4 Autistic Disorder, 8 AS, 3 PDD-NOS)	DSM-IV, ADI-R, ADOS-G	Age, IQ	fMRI task of attention to gaze and arrows
Yan (2018)	531 total	u/k	u/k	ABIDE dataset, various	ABIDE dataset, various	Age, sex	Analysis of regional HRF differences and functional connectivity

							MRI
Yang (2018b)	10:6	10.44 (2.83)	45.25 (1.00)	ASD	Clinical Diagnosis against DSM-IV	Age, sex	Voxel-based morphometry and diffusion tensor imaging.

Note. ABIDE, Autism Brain Imaging Data Exchange; ADI-R, Autism Diagnostic Interview, Revised; ADOS, Autism Diagnostic Observation Schedule; ADOS-G, ADOS-General; AS, Asperger Syndrome; ASC, autism spectrum condition; ASD, autism spectrum disorder; CARS, Childhood Autism Rating Scale; CPT, continuous performance test; DSM-IV, Diagnostic and Statistical Manual of the American Psychiatric Association, Fourth Edition; FSIQ, full-scale IQ; HFA, high-functioning autism; PDD-NOS, pervasive developmental disorder, not otherwise specified.

Appendix 4: McKechanie *et al*, Genes (2019)

Autism in fragile X syndrome; a functional MRI study of facial emotion-processing

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Article

Autism in Fragile X Syndrome; A Functional MRI Study of Facial Emotion-Processing

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Abstract: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism spectrum disorder, and among those with fragile X syndrome, approximately 1/3rd meet a threshold for an autism spectrum disorder (ASD) diagnosis. Previous functional imaging studies of fragile X syndrome have typically focused on those with fragile X syndrome compared to either neurotypical or autism spectrum disorder control groups. Further, the majority of previous studies have tended to focus on those who are more intellectually able than is typical for fragile X syndrome. In this study, we examine the impact of autistic traits in individuals with fragile X syndrome on a paradigm looking at facial emotion processing. The study included 17 individuals with fragile X syndrome, of whom 10 met criteria for autism as measured by the Autism Diagnostic Observation Schedule (ADOS). Prior to the scan, participants rehearsed on a mock scanner to help acclimatize to the scanner environment and thus allow more severely affected individuals to participate. The task examined the blood-oxygen-level-dependent (BOLD) response to fearful and neutral faces taken from the Ekman faces series. Individuals in the autism group had a region of significantly reduced activity centered on the left superior temporal gyrus, compared to those with FXS alone, in response to the fearful faces. We suggest that autism in individuals with fragile X syndrome is associated with similar changes in the neurobiology of facial emotion processing as seen in idiopathic autism.

Keywords: fragile X syndrome; autism; functional imaging; emotion-processing

1. Introduction

Fragile X syndrome (FXS) is a neurodevelopmental disorder occurring in approximately 1 in 3000–4000 males and 1 in 6000–8000 females and is the leading inherited cause of intellectual disability [1]. Further, a significant proportion of individuals with FXS meet diagnostic criteria for autism, with FXS being the leading monogenic cause of autism.

It was recognized in some of the early described series of confirmed individuals with fragile X syndrome that there were higher levels of autistic traits, by way of social, communication, and sensory difficulties; than could be accounted for by level of intellectual disability alone [2–7]. In parallel, early studies in which groups with autism were screened for fragile X reported that up to 16% of autistic males had fragile X syndrome [8–13]. With autism now being more widely recognized, especially in those without an intellectual disability, these estimates are consequently lower, with fragile X syndrome accounting for approximately 0.5% of individuals with autism [14–16]. Despite the association between autism and fragile X syndrome, in many cases, the presentation of autism in the context of FXS differs subtly, but importantly, from the prototypical presentation in idiopathic autism [17] and it is not clear to what extent the autistic traits reported in FXS are the result of the

same underlying process as those observed in idiopathic autism [18]. Whereas fragile X syndrome is a genetically-defined condition with a common phenotype, albeit with some variation in presentation, and an increased prevalence of the disorder; autism itself is a condition defined by its behavioral phenotype and with a wide variety of aetiologies, both known and unknown. Thus, as entities, they are categorically different, a theme previously investigated and discussed by Hall et al. [19]. Of note, is that discussion about whether autism in FXS is the same as idiopathic autism, is paralleled beyond FXS, with the validity of the idea of autism across the spectrum representing the same entity being questioned, particularly in the light of the revisions on ASD incorporated into DSM-5. This issue is far from new; indeed, even in the early years of autism research, Kanner was bemoaning the same issue; feeling that his idea of a relatively rare and pure entity was being challenged, noting that others appeared to wish to throw "diagnostic criteria to the winds"[20]. Notwithstanding this issue, given the increased co-occurrence of autism in FXS, the question of the nature of the overlap remains of interest.

One of the central features of autism is a difference in reciprocal social communication and interaction. What underlies this from a biological basis has been the basis of a number of theories. Whilst this likely varies across the various aetiologies of autism; given the clustering of features that define autism, we may expect some shared underlying biology. One possible contributing factor is a differential perception of facial emotional stimuli in autistic individuals, which may then contribute to differences in social understanding, communication, and interaction. Whilst the majority of studies show diminished facial emotion recognition in autistic individuals, there is significant variability in the findings; with heterogeneity in study paradigms likely to explain at least part of this [21]. The time it takes for emotion recognition may also be an important difference [22], with individuals with autism typically taking longer to recognize the emotion [23]. It should also be noted that the direction of the relationship between facial emotion recognition and the impairments in social interaction typical of autism is not entirely clear: diminished social interaction is likely to give less exposure to facial stimuli and therefore interfere with development of the associated neural circuitry; whilst primary difficulties in facial emotion recognition may make social interaction difficult [24]. Meta-analyses of emotion processing functional imaging studies in autism show recruitment of different brain regions during facial emotion recognition, with regions of both hypo- and hyper-activation seen [25,26]. Of these, the strongest and most consistent findings have been differences in activation in the fusiform face area (FFA) and temporal structures. In the FFA, typically hypoactivation is seen in individuals with autism [27–31]. In their review of studies reporting on FFA activation, Perlman reports that this FFA hypoactivation was seen in two-thirds of studies, with equal activation seen in the remainder [31]. With regards to the results in temporal structures, both hypo- and hyper-activation have been reported [25,28,29,32,33], largely with a focus on the superior temporal gyrus and the superior temporal sulcus.

Functional MRI imaging has been used in fragile X syndrome quite extensively to try and better understand some of the key differences across individuals in a variety of domains, including facial/emotion/gaze processing [34–40], auditory processing [41], cognitive functions (memory, attention, cognitive interference, equivalence processing, arithmetic processing) [42–49], and functional connectivity [50,51]. Interestingly, most of these studies have examined groups of individuals with FXS of a mean age of 18 or below. It is important to consider that development continues throughout adulthood, and differences may either emerge or diminish over time. As the field of research develops, replications of these studies, as well as our own, in older adults would help to shine a further light on the developmental trajectories and maturing brain in FXS.

The previous functional imaging studies of face and gaze processing in fragile X syndrome have produced relatively heterogeneous findings [34–40]. As with the autism literature, this has likely been the result of a combination of factors, including: imaging paradigm used, balance of gender, level of intellectual functioning of the FXS group, and choice of comparison group. Of particular note is that given the relationship (albeit not direct) between intelligence quotient (IQ) and fragile X mental retardation protein (FMRP) levels [52], it is likely that at least some of the variability will be explained by the wide range of group mean IQ (61–91) of the individuals in these studies, and thus the likely

underlying FMRP levels. Of the 100 participants in these previous studies, there were 57 females and 43 males. Given that FXS is approximately twice as common in males as it is in females, this ratio of male to female participants, likely represents somewhat of a selection bias for females. These factors, at least in part, likely reflect the significant difficulties in recruiting and scanning individuals with more significant intellectual impairment, who are more likely to be male.

In the fragile X syndrome studies of emotion processing concerned with individuals of mean IQ <70 (i.e., considered to have an intellectual disability; a key feature of the full fragile X syndrome), the results showed decreased prefrontal activation in FXS compared to typically-developing (TD) controls [38,40]; increased left insula activation in FXS compared to TD controls [40]; left frontal gyrus hypoactivation in FXS compared to TD controls [35]; and increased activation in left hippocampus, left superior temporal gyrus, right insula, and left postcentral gyrus in FXS compared to TD and ASD controls [35]. In the study of neural habituation to faces, the FXS group showed significant sensitization and decreased habituation in cingulate gyrus, fusiform gyrus, and frontal cortex compared to IQ and autism-matched controls [34].

In this study, we aimed to further explore the relationship between autism and emotion-processing in individuals with fragile X syndrome. In particular, we were interested in whether the same patterns of differential neural activation during emotion-processing seen in individuals with idiopathic autism compared to typically-developing controls, would be seen in a group with FXS + autism, compared to individuals with FXS alone. Our hypothesis was that we would see reduced activation in the FFA and altered activity in superior temporal structures in the FXS + autism group.

2. Materials and Methods

2.1. Participant Recuritment

Initial recruitment was through the Fragile X Registry at The Patrick Wild Centre in Edinburgh. With the support of the UK-based family support charity, The Fragile X Society, the study was also advertised in their quarterly print newsletter and on their website. Subsequently, information sheets and letters of invitation were sent out to families registered with the society as being interested in research. Ethical permission for the study was granted by the National Research Ethics Service Scotland A Research Ethics Committee (reference 12-SS-0117).

2.2. Imaging Procedure

Prior to their scan, participants were given the opportunity to rehearse the scanning procedure on two mock scanners available. The first mock scanner had been built in the Patrick Wild Centre for a previous study to facilitate desensitization to the scanning procedure, and was used extensively in this study for rehearsal and acclimatization. A further mock scanner, housed in the Clinical Research Imaging Centre (CRIC) was also used, and participants were able to rehearse on this immediately prior to their main scan. This mock scanner was a replica of the main scanner used, with the only difference being that it did not have a main coil. However, the use of earplugs, headphones, and an audio recording of the scanning sequences used in the main scanner all helped to simulate the sensory experience. Only when participants were comfortable in the mock scanner did they proceed to the main scan. Eight individuals did not successfully proceed beyond the mock scanning stage. See Figure A1 (Appendix B) for details.

2.3. Imaging Sequences

All scans were completed on a Siemens MAGNETOM Verio 3T scanner. For the structural imaging, using an MPRAGE sequence, a T1 structural image was obtained made up of 160 coronal slices of 1 mm slice thickness and 1 mm × 1 m × 1 mm voxels. A repetition time (TR) of 2.3 s, an echo time (TE) of 2.98 ms, flip angle of 9°, and field of view (FOV) of 256 mm were used. For the functional imaging, 159 volumes were acquired; each containing 26, interleaved, 5 mm slices of voxels 3.4 mm × 3.4 mm × 5 mm. In this case a TR of 1.56 s, a TE of 26 ms, flip angle of 66° and FOV of 220 mm were used.

The functional imaging task used was a block-design task with two main conditions, including a series of neutral faces, and a series of fearful faces, the faces being taken from the Pictures of Facial Affect series [53]. We used the fearful and neutral stimuli as differences in the processing of fearful stimuli have been shown to be particularly affected in autism [33].

A visual fixation cross was presented at the beginning and end of the sequence, as well as between the conditions of interest. In the contrasts comparing against baseline, the fixation cross was considered as the baseline condition. The complete sequence presented six blocks, each of six faces alternating between blocks of fearful or neutral faces. Within each block, each face was shown for 3.5 s with an inter-stimulus interval of 0.5 s. In between each block was an interval of 12.5 s during which a fixation cross was shown. There were two variations of the sequence, with one starting with a block of neutral faces and the other starting with a block of fearful faces; these sequences being balanced across the groups. As had been rehearsed in the mock scanners, participants were asked to depress a trigger button each time they saw an image. This was principally used as an in-scan method for ensuring participants were attending to the task, with participants needing to respond successfully on more than 80% of faces to be included in further analysis.

2.4. Image Processing and Analysis

2.4.1. Preprocessing of fMRI Data

Images were processed and analysed using the Statistical Parametric Mapping (SPM) program (version 12, Functional Imaging Laboratory, Wellcome Trust Centre for Human Neuroimaging, University College London, London, UK; fil.ion.ucl.ac.uk/spm/) running within Matlab (R2011b (version 7.13.0.564), MathWorks, Natick, MA, USA). The ArtRepair toolbox version 5b3 [54] for SPM was used to analyze and repair motion artefacts using the single subject pipeline described by Mazaika [55]. Full details of the preprocessing pipeline are contained in Appendix A.

2.4.2. Statistical Analysis of fMRI Data

For each contrast examined, a design matrix was created incorporating weightings for the neutral and fear conditions. A 128-s high-pass filter was used to remove slow signal drifts. Second-level analyses were generated using these first level contrast images for each participant to consider differences in activation, both within groups and between groups. The initial height threshold was set at $p < 0.001$ uncorrected with results considered significant at $p < 0.05$ at cluster level after family-wise error correction. Age was included as a covariate of no interest in the between-group analyses given the trend towards a significant difference between the two groups on age.

2.5. Measure of Cognitive Ability

The Kaufman Brief Intelligence Test (K-BIT) was used with all participants as a measure of cognitive ability. The K-BIT comprises of three sub-tests (Verbal Knowledge, Riddles, and Matrices) and takes approximately 20 min to complete [56], giving verbal, performance, and composite IQ scores.

2.6. Measure of Autistic Traits

The Autism Diagnostic Observation Schedule-2 (ADOS-2, henceforth, simply referred to as 'ADOS') was used to directly measure autistic traits. The ADOS is a semi-structured interview that uses a set of prescribed 'presses' to elicit, demonstrate, or create the space in which autistic features may be assessed either by the presence or absence of features that are useful in helping to establish an autism diagnosis [57]. This format allows for the assessment of autistic and associated features including 31 items across 5 domains. The 5 domains include the domains considered in autism diagnosis (social, communication, and stereotyped behaviors and restricted interests) plus the related domains of creativity and associated features.

For each participant, we used the cutoff of a combined social and communication total of ≥10 to divide the group into 'FXS' and 'FXS + autism' groups for the between-group analyses. Calibrated Severity Scores (CSS) were also calculated using the published algorithms, to provide a continuous measure of autistic traits for regression analysis. In the case of the participants who were scored on the ADOS module 4, the CSS algorithm subsequently published by Hus & Lord [58] was used.

3. Results

3.1. Feasibility of Functional Imaging in Fragile X Syndrome

Of the individuals who received the invitation to participate, or who saw the study advertisement, a total of 58 expressed interest. After discussion by telephone, 32 of these participants and their families attended an initial visit and trial on the mock scanner. Of those who did not progress from an expression of interest to a visit, a number of reasons were cited; however, for most it was the combination of all the barriers (principally distance, travel, and logistics) that were reported as being the reason not to progress. In many cases, these reasons should also be considered in the context of the participant's and family's uncertainty as to whether the participant would actually be able to successfully complete the scanning sequence. Of 32 participants who attended an initial visit and trial on the mock scanner, 22 attended the main scanner visit, 21 completed a structural scan, 18 completed a functional scan, and 17 were included in the analyses. Figure A1 (Appendix B) lays out the recruitment path from initial approach to completed scans.

As expected, the biggest dropout after attending an initial visit was between the mock scanner trial and the main scanner visit, emphasizing the screening role that the opportunity to rehearse on the mock scanner can provide. Whilst most of the participants had not had prior contact with the research team, a significant proportion (41%) had been involved with previous studies at The Patrick Wild Centre, and were already familiar with the staff, facilitating participation.

3.2. Investigating the Role of Autism in Mediating Facial Emotion Processing

To consider the relative impact of autistic traits on facial emotion processing, the data were examined in two separate ways. Firstly, by dividing the participants into two groups: those meeting the ADOS threshold for autism (social and communication total ≥10) and those not (social and communication total <10); contrasts were examined in SPM comparing the groups. Secondly, we calculated Calibrated Severity Scores (CSS) from the raw ADOS data using the published algorithms. These scores were then regressed against the contrast of interest within the autism group alone.

3.2.1. Between-Group Analyses

In these analyses, we compared the response to each contrast between the groups of those with FXS alone and those with FXS + threshold autism traits on the ADOS. The makeup of the two subgroups is shown in Table 1. In general, the use of prescription medication in the participants was low, with only four participants taking regular psychoactive medication. All four participants were taking mavoglurant (AFQ056, Novartis AG, Basel, Switzerland); one in the non-autism group and three in the autism group. We consider the relatively low use of psychoactive medications in the sample to likely represent a combination of prescribing practice in the U.K. and likely a degree of selection bias—that those who were more affected and thus more likely to be on medication, were less likely to be able to participate. Further, our sample was on average older than those in most studies. As such, whilst a number of participants had been on psychoactive medications as children; they were no longer on them. Epilepsy, whilst more common in FXS, was under-represented in this sample, with no participants being treated for epilepsy.

Table 1. Baseline details of participants included in the imaging analyses.

	Autism Group	Non-Autism Group
N	10	7
Male: female	8:2	5:2
Age	18 (6.2)	27 (11.7)
Full-scale IQ	59 (8.9)	63 (14.2)
Verbal IQ	69 (11.9)	71 (14.2)
Performance IQ	58 (11.1)	60 (14.4)
ADOS Total	15 (10–20)	2 (0–5)
ADOS CSS	8 (6–10)	2 (1–2)

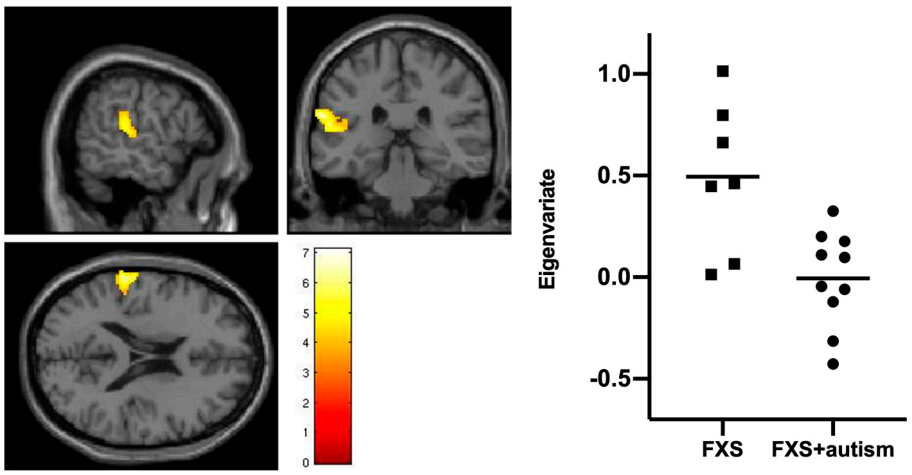
Results show group means (s.d.) for age and IQ and median (range) for the ADOS scores. The groups were not significantly different on gender ($p = 0.682$), age ($p = 0.092$), full-scale IQ ($p = 0.528$), verbal IQ ($p = 0.695$) or performance IQ ($p = 0.704$). ADOS, Autism Diagnostic Observation Schedule; CSS, Calibrated Severity Score.

In the analysis comparing response to fearful faces versus baseline, there was a region of significantly different activity between the groups centered on the left superior temporal gyrus (STG) and extending to the rolandic operculum and supramarginal gyrus. Specifically, this region showed significantly greater activation in the FXS group, compared to the FXS + autism group. Co-ordinates in Montreal Neurological Institute (MNI) space for this cluster are given in Table 2. The cluster is shown in Figure 1a and the extracted values of the Eigenvariates are plotted in Figure 1b. The cluster remains significant ($p_{FWE\text{-}corr} = 0.002$; $k_E = 511$; $Z_{=} = 4.52$; x, y, z = −64, −30, 22) when including medication use as a covariate.

Table 2. Region of significantly different response to fearful faces between FXS and FXS + autism groups.

Cluster	$p_{FWE\text{-}corr}$	k_E	$Z_{=}$	x	y	z
Left superior temporal gyrus	0.001	570	4.45	−64	−30	22

Significance given as cluster-level, familywise-error corrected value. x, y, z co-ordinates given in MNI space.



(**a**) (**b**)

Figure 1. (**a**) Cluster of significantly greater brain activation in the non-autism group, compared to the autism group during the fearful faces versus baseline contrast. Region projected on the canonical

single subject T1 image from SPM12. (**b**) Extracted Eigenvariate values in the two groups from the cluster shown in panel (**a**).

There were no clusters of significant difference between the groups when considering response to neutral faces vs. baseline, or on the more subtle contrast of fearful faces vs. neutral faces. Whilst we did see the expected activation in the fusiform face area in both groups in response to the facial stimuli (both fearful and neutral), there were no between-group differences found.

3.2.2. Correlation between ADOS Calibrated Severity Score and Response to Fearful and Neutral Faces

In this analysis, the calculated CSS score was regressed against response to each of: all faces, neutral faces, and fearful faces in the autism subgroup. There was a cluster of positive correlation between CSS score and the response to all faces, as reported in Table 3. The region is shown in Figures 2 and 3 shows the correlation between CSS and the extracted value of the cluster Eigenvariates.

Table 3. Region of significant correlation between ADOS Calibrated Severity Score and response to all facial stimuli.

Cluster	$p_{FWE\text{-}corr}$	k_E	$Z_{\equiv}$	x	y	z
Left cerebellum, anterior lobe, lobules IV/V	0.029	198	4.01	−24	−42	−34

Significance given as cluster-level, familywise-error corrected value. x, y, z co-ordinates given in MNI space.

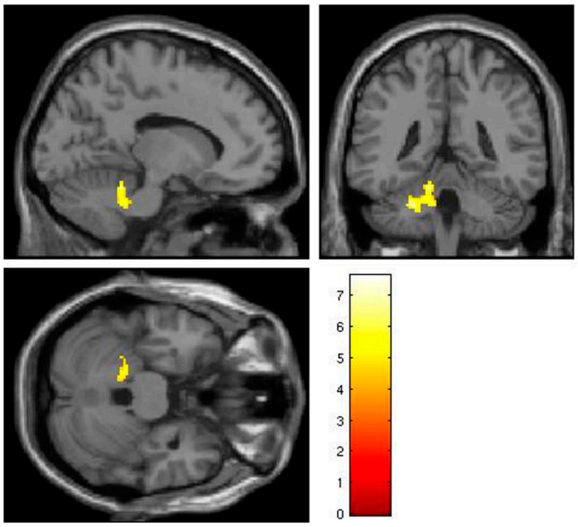


Figure 2. Cluster of activation significantly correlated with ADOS Calibrated Severity Score in the autism subgroup. Region projected on the canonical single subject T1 image from SPM12.

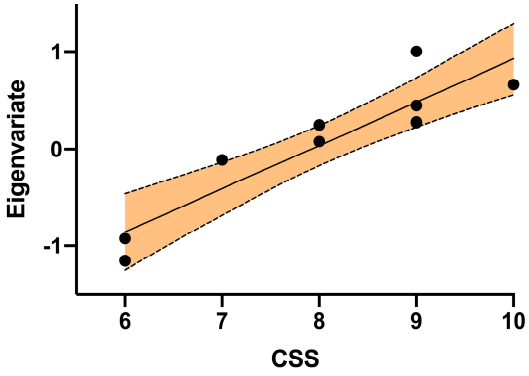


Figure 3. Scatter plot of the correlation between extracted Eigenvariate value and Calibrated Severity Score (CSS) from the cluster shown in Figure 2. The solid line shows the linear regression, with the dotted lines demarcating the 95% confidence bands.

While there was no significant correlation to either of the neutral > baseline or fear > baseline contrasts at a whole-brain level, a small volume correction (SVC) centered on the area of significant activation for the faces > baseline contrast was used to investigate which contrast was driving the faces > baseline result. Using this SVC, an area of significant activation was identified in the neutral > baseline contrast, whereas there was no significant activation in this area under the fear > baseline contrast. The results are shown in Table 4.

Table 4. Region of significant correlation between ADOS Calibrated Severity Score and response to neutral facial stimuli using the small volume correction described.

Cluster	$p_{FWE\text{-}corr}$	k_E	$Z_{\equiv}$	x	y	z
Left cerebellum, anterior lobe, lobules IV/V	0.006	27	3.70	−20	−38	−30

Significance given as cluster-level familywise-error corrected value. x, y, z co-ordinates given in MNI space.

4. Discussion

In this study, we explored the role of autism in facial emotion processing in individuals with fragile X syndrome. Previous functional imaging studies of facial emotion processing in both autism and FXS had suggested that differences in activation in the fusiform face area and the superior temporal gyrus were the most robust findings. Interestingly, whilst we did elicit significant FFA activation at a whole group level, we did not detect any differences between the groups.

However, our finding of significantly reduced activation in the left superior temporal gyrus (STG) / superior temporal sulcus (STS) in those with FXS+ autism compared to those with FXS alone overlaps the previous findings in individuals with idiopathic autism [26]. Our result also replicates the finding of Dalton of increased activity in the same region in individuals with FXS compared to both typically-developing and autism controls [35]. Interestingly, in the FXS group reported by Dalton, none of them had a clinical diagnosis of autism, and the group had relatively low average autistic traits, as measured by the Social Communication Questionnaire (SCQ) (mean SCQ of 9). Thus, we suggest that the FXS group studied by Dalton is likely to be comparable to our non-autism FXS group. Taken all together, the result suggests that autism, whether idiopathic or associated with FXS, may be associated with the same impact on the neurological underpinnings of facial emotion processing.

Our finding in the cerebellum of a correlation between activation to neutral faces and CSS scores is interesting in that the findings for the role of the cerebellum in social processing in autism have

generally been that cerebellar activation is diminished in individuals with autism compared to typically-developing controls [59–62]. However, in their meta-analysis of 350 fMRI studies examining the role of the cerebellum and social cognition, Van Overwalle et al. suggest that cerebellar activity may actually increase when the level of abstraction in the task increases, and with it the demand on executive resource [63]. If neutral faces are considered to be more ambiguous and thus may appear more abstract than overtly emotional (in this case, fearful) faces, then this may be an explanation for the finding. However, given that this finding is largely in contrast to the literature, we think it only appropriate to be circumspect with regard to this finding. Further, given its level of significance at a whole brain level, we can be less confident in it. Indeed, correcting for multiple comparisons, the result would no longer be significant.

In the between-group comparisons, we were looking at three main contrasts: response to neutral faces versus baseline, response to fearful faces versus baseline, and response to fearful faces versus neutral faces. Although the only significant differences that we noted were in the fearful versus baseline contrast, as noted in the results, we did not find any group differences on the fearful versus neutral contrasts. We therefore cannot specifically ascribe our findings to the processing of emotional content per se and it is possible that they relate to more general face processing differences.

Beyond the findings from our imaging analysis, we also describe the methods used in successfully imaging a cohort of individuals with fragile X syndrome who were more intellectually impaired than many participants in prior studies. The mean full scale IQ of individuals in this study was 60.9, while images were successfully acquired in individuals with IQ as low as 40. Using these methods, we have, therefore, shown that it is possible to successfully image people with FXS and significant ID. We hope that the descriptions of methods used and the description of where participants dropped out from the study will be of use to other researchers in planning similar studies.

4.1. Limitations

4.1.1. Participants

The study has a number of limitations. Firstly, as with many of the previous imaging studies in fragile X syndrome, larger numbers would have provided more power to detect more subtle group differences. Nonetheless, this is still one of the larger functional imaging study of males with FXS and we hope that it can add to the literature. Acknowledging the difficulties in recruiting the most severely affected individuals, there was likely a degree of selection bias; with a number of potential participants either not responding to the invitations, or dropping out once they had tried the mock scanner. In terms of comparison or control groups, previous studies in FXS have taken a variety of approaches: typically-developing controls, ASD controls, typically-developing developmental age-matched controls or developmental delay controls. In this study, we were interested in the effect of autism on individuals with FXS and thus chose to compare two groups of individuals with FXS; one with high autistic features, and one without. It would have been interesting to also include further comparison groups; however, that was beyond the scope of this study, although is a focus of currently ongoing work.

4.1.2. Measures

The ADOS, whilst commonly used, is not validated for use in adults with such significant intellectual disability; although the bimodal distribution of both ADOS total and CSS scores gives a degree of confidence that our groups represented individuals with significant differences in social communication and interaction. Had we chosen the lower threshold of ≥7 for autism spectrum disorder as published in the ADOS, we would still have had groups of the same makeup. Further, the ADOS was completed by two researchers (A.G.M. (consultant psychiatrist) & S.C. (clinical & research psychologist)) who are trained as research-reliable ADOS users, adding to the degree of reliability that can be afforded to the findings. A full clinical workup considering autism diagnosis, would of course have been preferable, however, was not feasible within the constraints of the study.

4.1.3. fMRI Paradigm and Acquisition

During the acquisition of the imaging data, we used a trigger for the participants to indicate when they had seen a face, with participants needing to respond successfully on more than 80% of faces to be included in further analysis. However, our scanning facility unfortunately did not have in-scanner eye-tracking available, and as such we do not know for how long, or with what pattern, the participants visually attended to the stimuli. Given that both FXS and autism are associated with gaze aversion, and that there may be a group difference on gaze, we cannot be confident that our results do not represent differences in gaze, either instead of, or as well as, differences in underlying neural processing. The use of a fixation cross as baseline also means that we cannot attribute our findings specifically to face processing, as opposed to more general visual perceptual differences between the groups. Future research should include eye-tracking combined with a higher-level baseline, such as a scrambled face, to address this.

5. Conclusions

In this study, we have shown that autism in individuals with fragile X syndrome is associated with the same reduction in activation in the left superior temporal gyrus, as is seen in individuals with both idiopathic autism, compared to typically-developing controls; and in idiopathic autism, compared to non-autistic individuals with FXS. This supports the idea that autism in FXS may, at least in part, represent a good model for autism more broadly.

Author Contributions: Conceptualization, A.G.M and A.C.S.; methodology, A.G.M; analysis, A.G.M; investigation, A.G.M., S.C., S.E.A.E. and A.C.S.; writing—original draft preparation, A.G.M; writing—review and editing, A.G.M., S.C., S.E.A.E. and A.C.S.; project administration, A.G.M; funding acquisition, A.G.M.

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Appendix A

Images were analyzed using the Statistical Parametric Mapping (SPM) program (version 12, Functional Imaging Laboratory, Wellcome Trust Centre for Human Neuroimaging, University College London, London, UK; fil.ion.ucl.ac.uk/spm/) running within Matlab (R2011b (version 7.13.0.564), MathWorks, Natick, MA, USA). Data were initially reconstructed using the DICOM Import function within SPM for further processing within SPM.

Prior to pre-processing, the first seven volumes of the functional scans were discarded to reduce the impact of T1 equilibrium effects. Images were initially realigned to the mean EPI image using the Realign (realign and unwarp) module of SPM. The T1 structural image was co-registered to the mean EPI image. The images were realigned, before being smoothed with a 4 mm FWHM Gaussian smoothing kernel. The ArtRepair toolbox version 5b3 [54] for SPM was used to first examine and then repair the volumes, using the *art_motionregress* and *art_global* modules of the single subject pipeline described by Mazaika [55] The *art_motionregress* algorithm of ArtRepair was used as an alternative to adding the movement parameters in the design matrix, as the motion regressors have been described as not being sufficiently accurate to account for the relatively larger movements of clinical subjects

[64]. The images were subsequently analyzed and repaired using the *art_global* module in ArtRepair. In this step, the volumes were examined for fast head movements, and volumes with movement of >0.5 mm/TR were interpolated with the nearest usable volumes. 10/17 scans had to be repaired in this way. Where a scan had >20% of volumes with >0.5 mm/TR movement, the scan was excluded. Only 1 scan had to be excluded for this reason. The T1 structural image was segmented before both structural and functional images were normalized using normalization parameters arising from the T1 image segmentation. Finally, a 7 mm kernel was used in a second smoothing step; the combination of 4 mm and 7 mm smoothing being approximately equivalent to the commonly-used single 8 mm kernel [64].

Appendix B

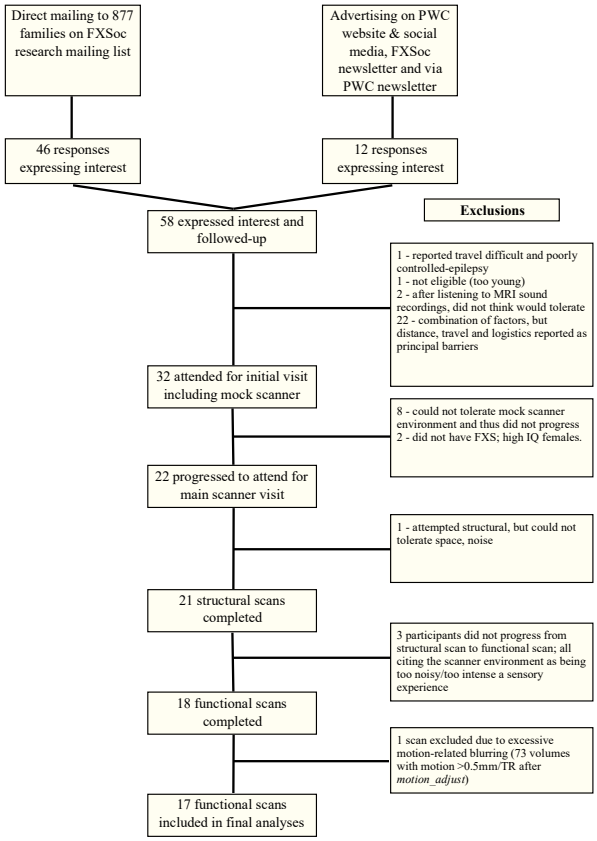

Figure A1. CONSORT diagram of recruitment and scanning. FXSoc—Fragile X Society.

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Appendix 5: Results of faces > baseline contrast in SEN imaging study

Contrast: all faces > baseline

This contrast shows the overall brain activation associated with viewing faces (both neutral and fearful) compared to baseline.

Within-group results

Non-ASD group

The non-ASD group showed a large region of significant activation, with the supra-threshold peaks of activation in the left lingual gyrus and left cuneus.

Table A2

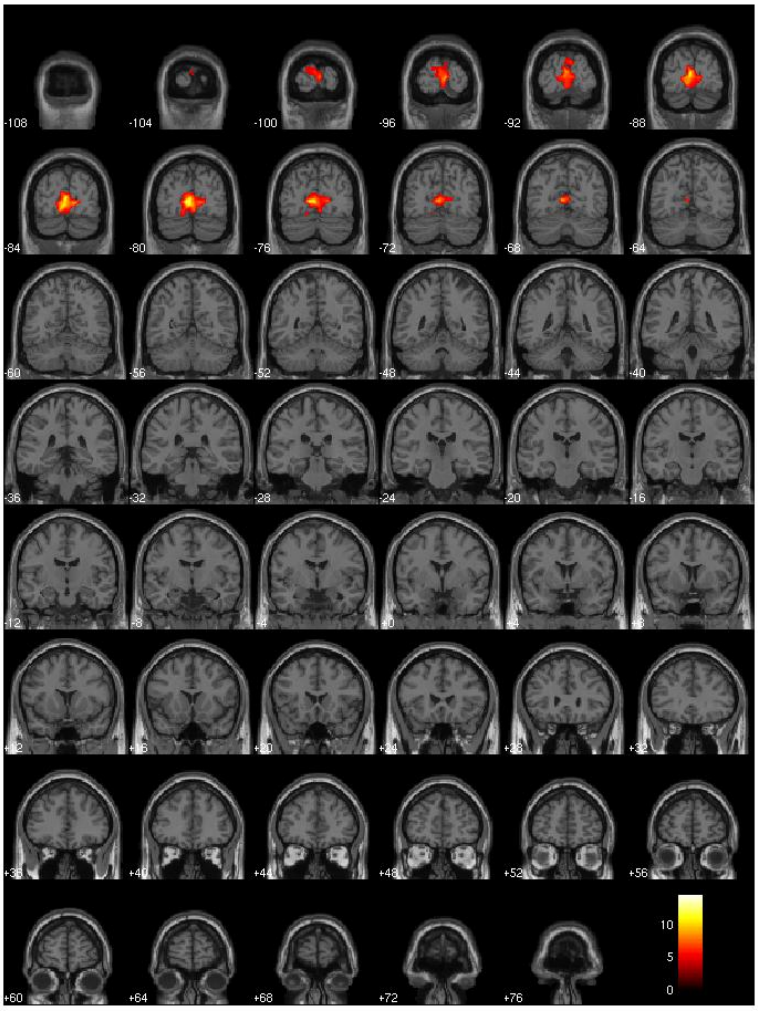
Clusters Of Brain Activation In Non-ASD Group During Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left lingual gyrus	<0.001	2225	5.03	-6	-78	4
Left cuneus				-2	-86	8
				-4	-70	6

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A1

Clusters Of Brain Activation In The Non-ASD Group During Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

ASD group

The ASD group showed a cluster of significant activation, with supra-threshold peaks of activation in the left fusiform gyrus and right cuneus and left declive. There was also a smaller cluster of significant activation centred in the right fusiform gyrus.

Table A3

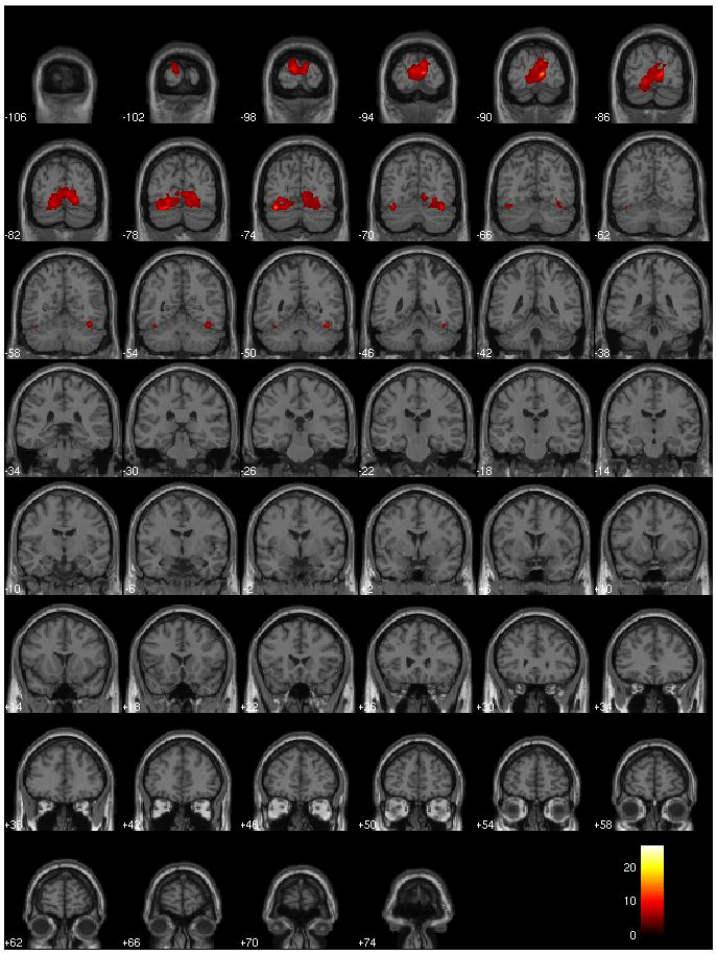
Clusters Of Brain Activation In ASD Group During Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Left fusiform gyrus	<0.001	3470	5.85	-34	-76	-20
Right cuneus				12	-90	4
Left declive				-22	-76	-20
Right fusiform gyrus	0.064	118	4.54	42	-48	-20
				40	-56	-18

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A2

Clusters Of Brain Activation In ASD Group During Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Between-group results

Non-ASD > ASD

The non-ASD group showed two clusters of significantly greater activation than the ASD group in the faces versus baseline contrast; one cluster centring in the left superior frontal gyrus, and the other centring on the left inferior parietal lobule.

Table A4

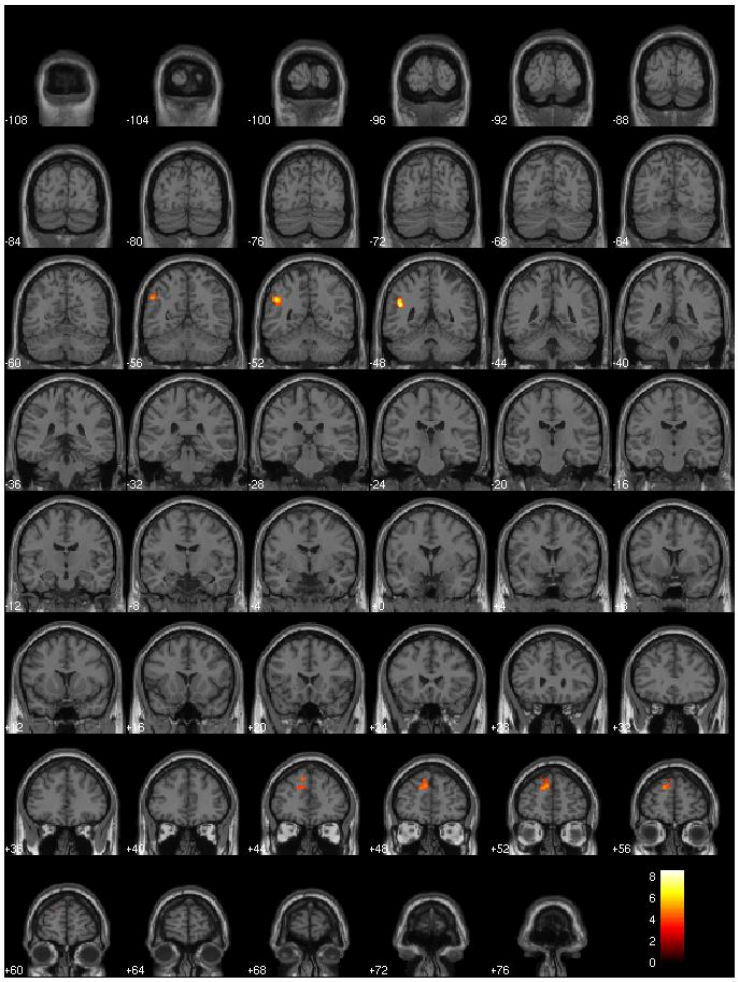
Clusters Of Greater Brain Activation In Non-ASD Group Compared To The ASD During The Faces Versus Baseline Contrast.

Cluster	pFWE-corr	kE	(Z≡)	x	y	z
Left superior frontal gyrus Left medial frontal gyrus	0.014	232	4.02	-12 -8 -12	54 44 42	28 42 28
Left inferior parietal lobule Left angular / supramarginal gyrus	0.045	177	5.17	-44 -48	-48 -52	24 32

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A3

Clusters Of Greater Brain Activation In Non-ASD Group Compared To The ASD During The Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

ASD>Non-ASD

The ASD group showed two clusters of significantly greater activation than the non-ASD group in the faces versus baseline contrast; one cluster centring in the right rolandic operculum, and the other centring on the left postcentral gyrus.

Table A5

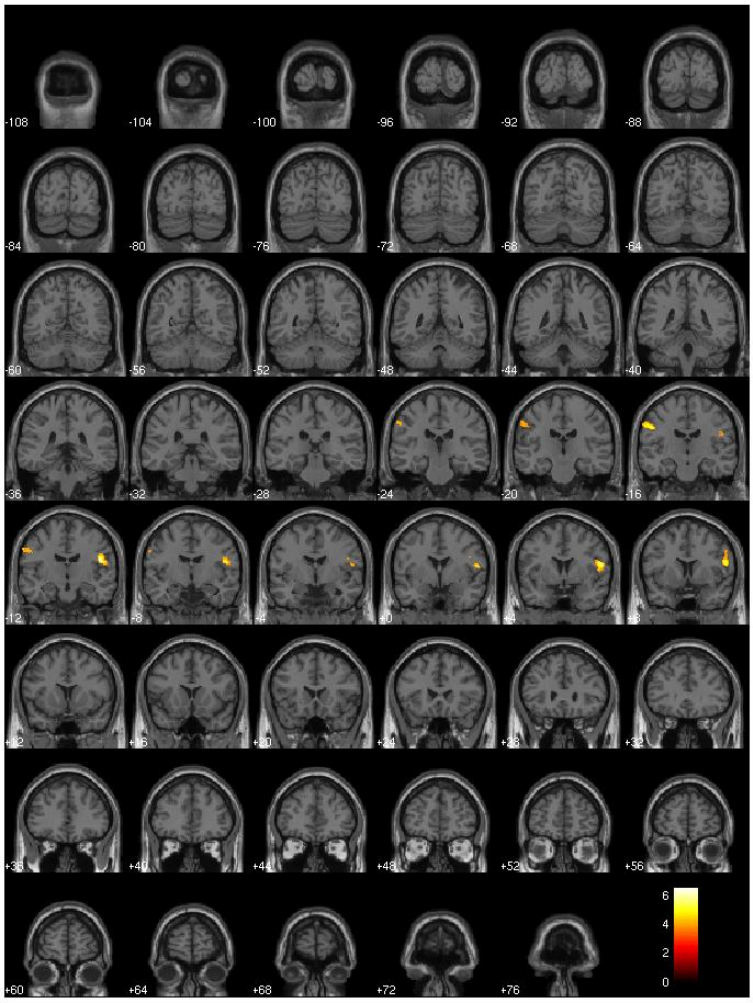
Clusters Of Greater Brain Activation In The ASD Group Compared To The Non-ASD Group During The Faces Versus Baseline Contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_=$)	x	y	z
Right rolandic operculum	<0.001	343	4.47	48	-12	22
Right inferior frontal gyrus				54	6	16
				54	6	6
Left postcentral gyrus	0.041	181	3.94	-50	-16	34
Left inferior parietal lobule				-56	-24	40

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A4

Clusters Of Greater Brain Activation In The ASD Group Compared To The Non-ASD Group During The Faces Versus Baseline Contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Table A6

Clusters Of Greater Brain Activation In The ASD Group Compared To The Non-ASD Group During The All Faces Versus Baseline Contrast Using A Small Volume Correction For Results From Philip et al (2012) ALE Meta-analysis.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{=}$)	x	y	Z
Right rolandic operculum	0.023	12	3.52	54	6	6

Note. Only clusters of $p<0.1$ family-wise error-corrected significance are reported.

Appendix 6: Results of faces > baseline contrast in FXS imaging study

Contrast: all faces > baseline

This contrast shows the overall brain activation associated with viewing faces (both neutral and fearful) compared to baseline.

Within-group results

Non-ASD group

The non-ASD group showed a large region of significant activation, with the supra-threshold peaks of activation in the right and left cuneus. There was also a smaller cluster of significant activation including the right fusiform gyrus.

Table A7

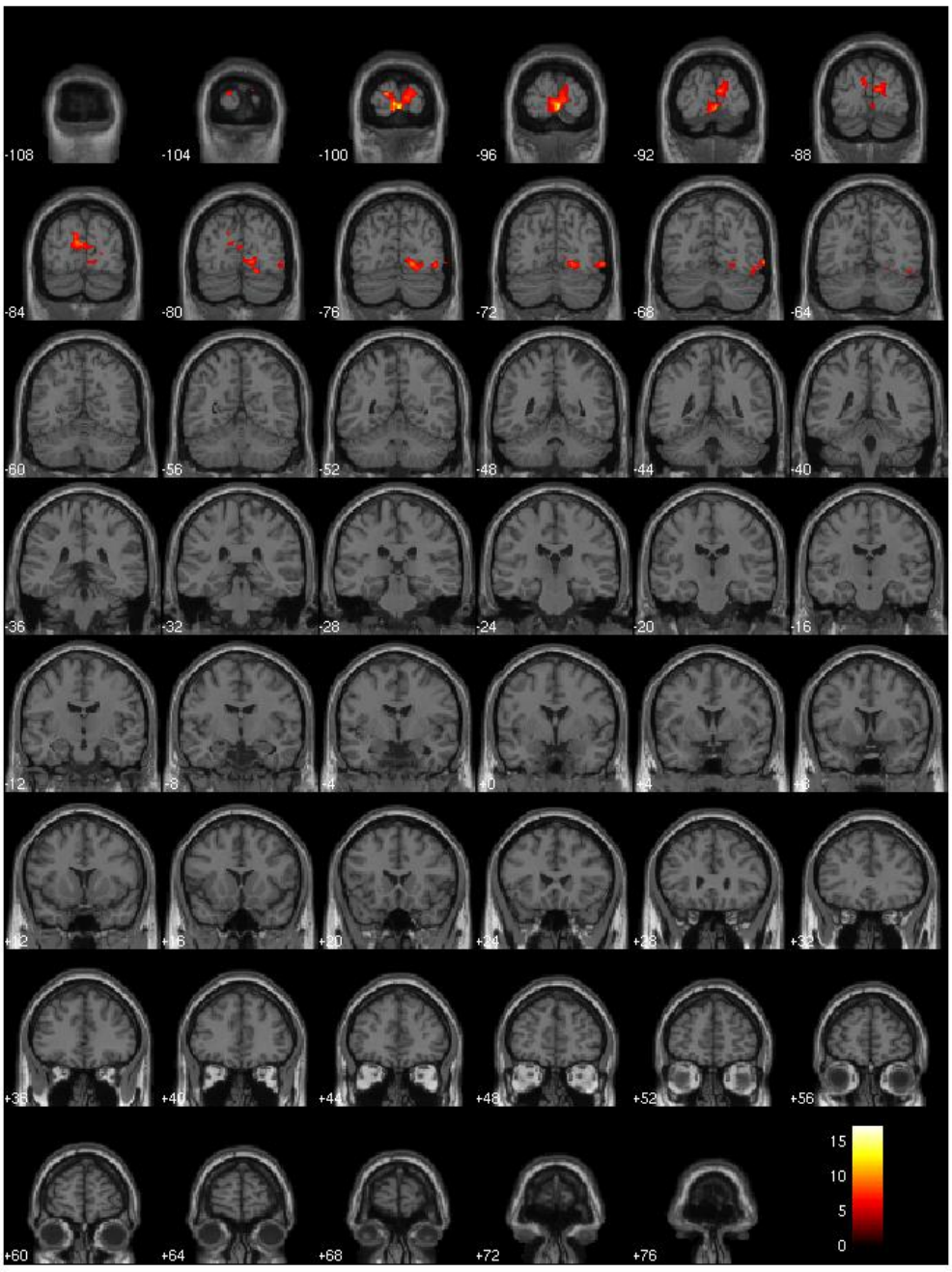
Clusters of brain activation in non-ASD group during faces versus baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_{\equiv}$)	x	y	z
Right cuneus	<0.001	1204	4.70	4	-98	-6
Left cuneus				-4	-100	-4
				-12	-102	8
Right inferior temporal lobe	0.048	145	4.31	54	-70	-10
Right inferior occipital lobe				44	-76	-8
				48	-66	-18
Right fusiform gyrus						

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A5

Clusters of brain activation in the non-ASD group during faces versus baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

ASD group

The ASD group showed a cluster of significant activation, with supra-threshold peaks of activation in the left cuneus.

Table A8

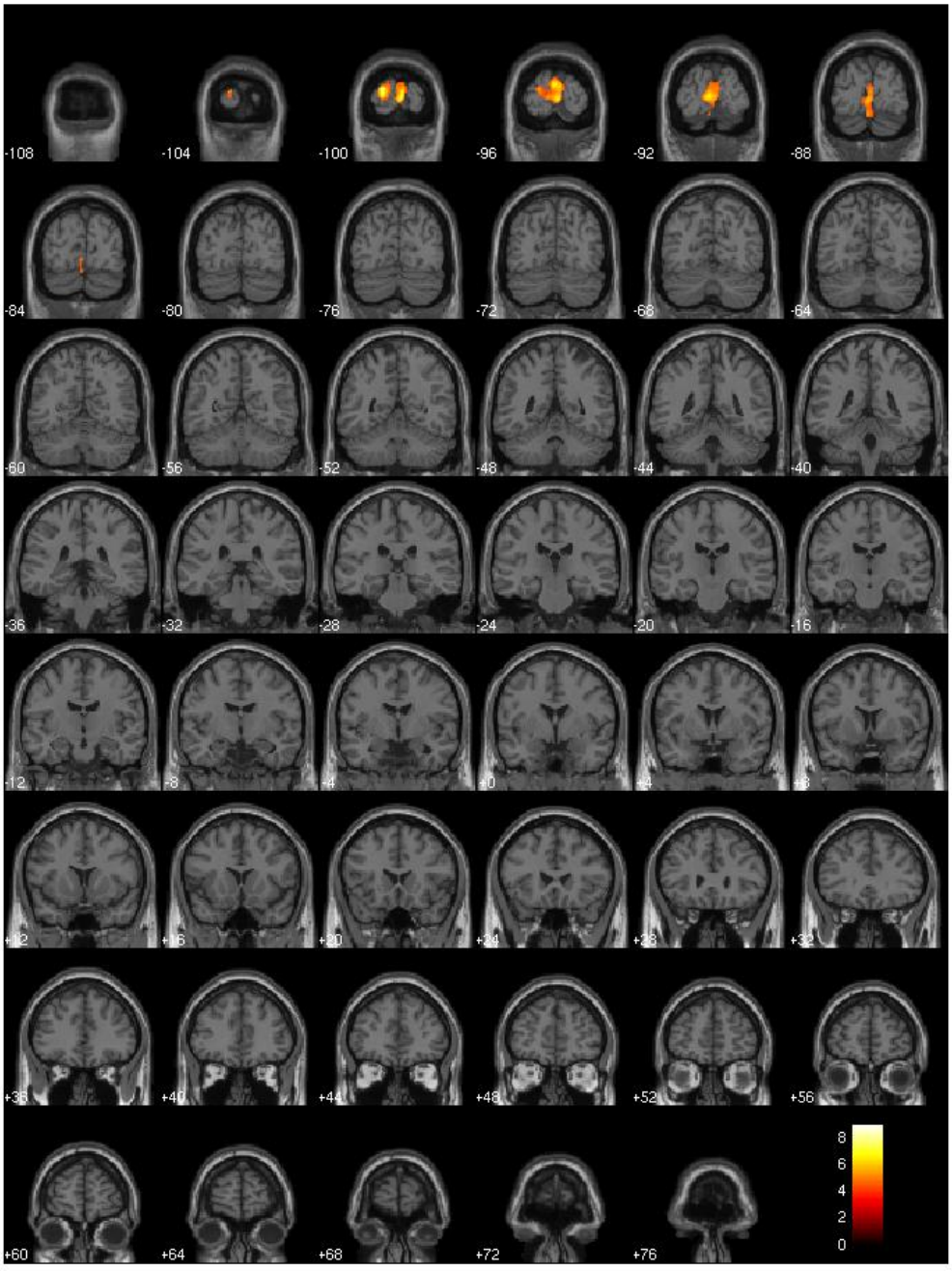
Clusters of brain activation in ASD group during faces versus baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	($Z_=$)	x	y	z
Left cuneus	<0.001	914	4.43	-16 6 0	-102 -100 -96	8 2 16

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A6

Clusters of brain activation in ASD group during faces versus baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Regression analyses

Within the autism group, there was a significant positive correlation between autism CSS score and activation to the faces > baseline contrast in a cluster in the left cerebellum, including lobules IV, V and VI.

Faces > baseline

Table A9

Cluster in the autism group on the faces versus baseline contrast with a significant positive correlation between brain activation and ADOS Calibrated Severity Score.

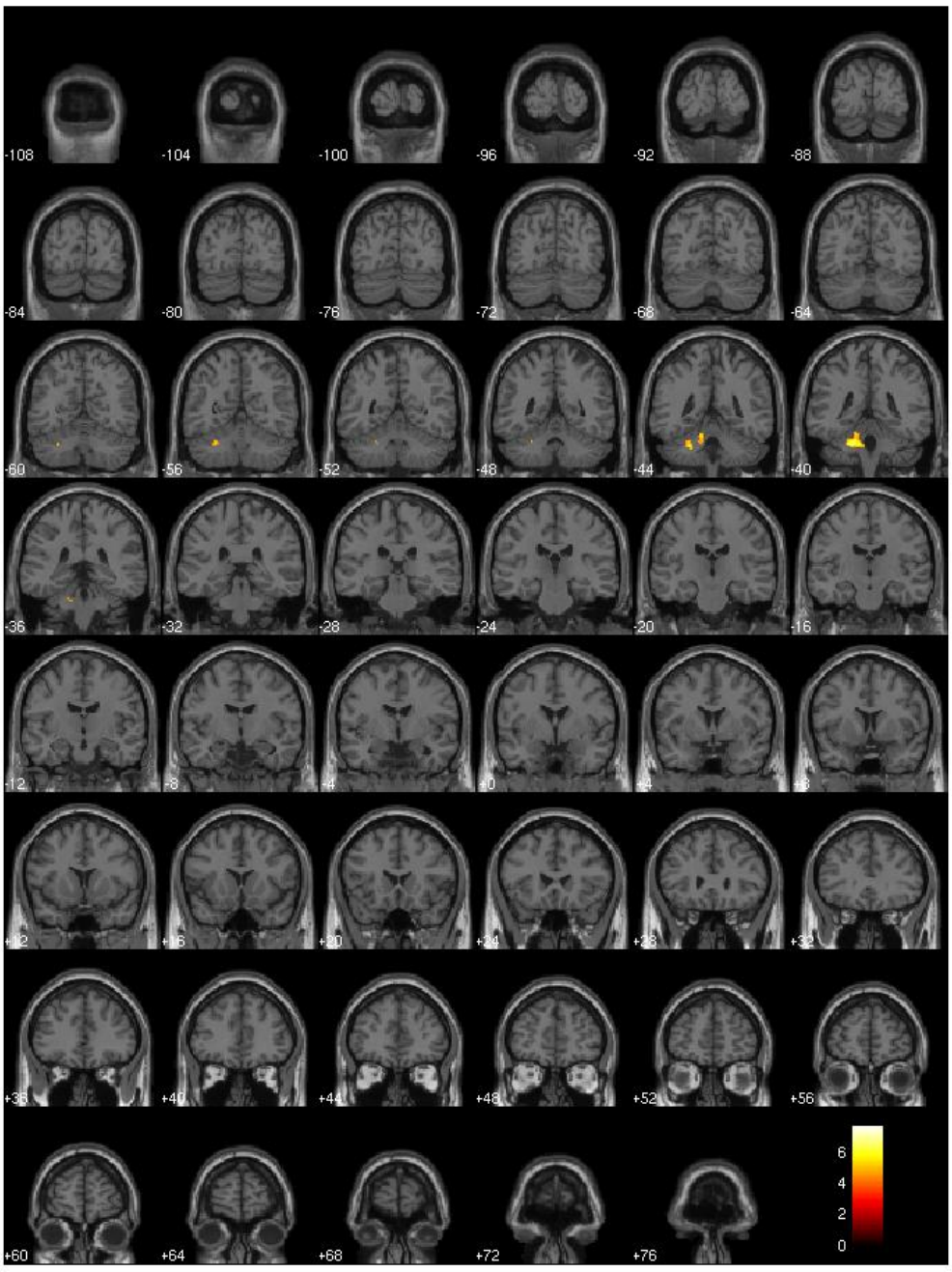
Cluster	$p_{FWE\text{-}corr}$	k_E	$(Z_{\equiv})$	x	y	z
Left cerebellum, lobules IV,V,VI	0.029	198	4.01	-24	-42	-34

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A7 shows the extent of the cluster and Figure A8 shows the extracted Eigenvariates from this cluster plotted against the CSS.

Figure A7

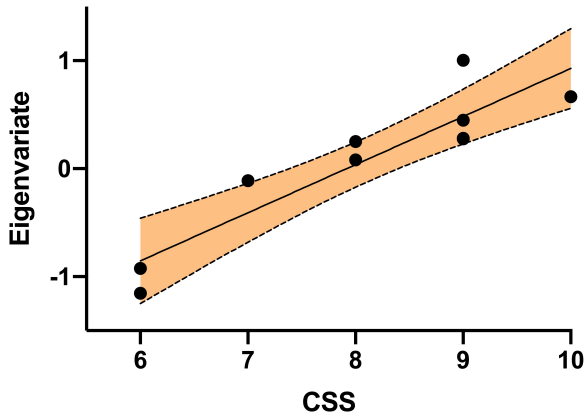
Clusters with a significant positive correlation between brain activation on the faces versus baseline contrast and an ADOS Calibrated Severity Score



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Figure A8

Scatter plot of the correlation between extracted Eigenvariate value and Calibrated Severity Score (CSS) from the cluster shown in Figure A7.



Note. Solid line shows the linear regression, with the dotted lines demarcating the 95% confidence bands.

Exploring the correlation between neural response and CSS score

While there was no significant correlation to either of the neutral > baseline or fear > baseline contrasts at a whole-brain level, a small volume correction centred on the area of significant activation for the faces > baseline contrast was used to investigate which contrast was driving the faces > baseline result. Using this SVC, an area of significant activation was identified in the neutral > baseline contrast, whereas there was no significant activation in this area under the fear > baseline contrast.

Neutral > baseline

Table A10

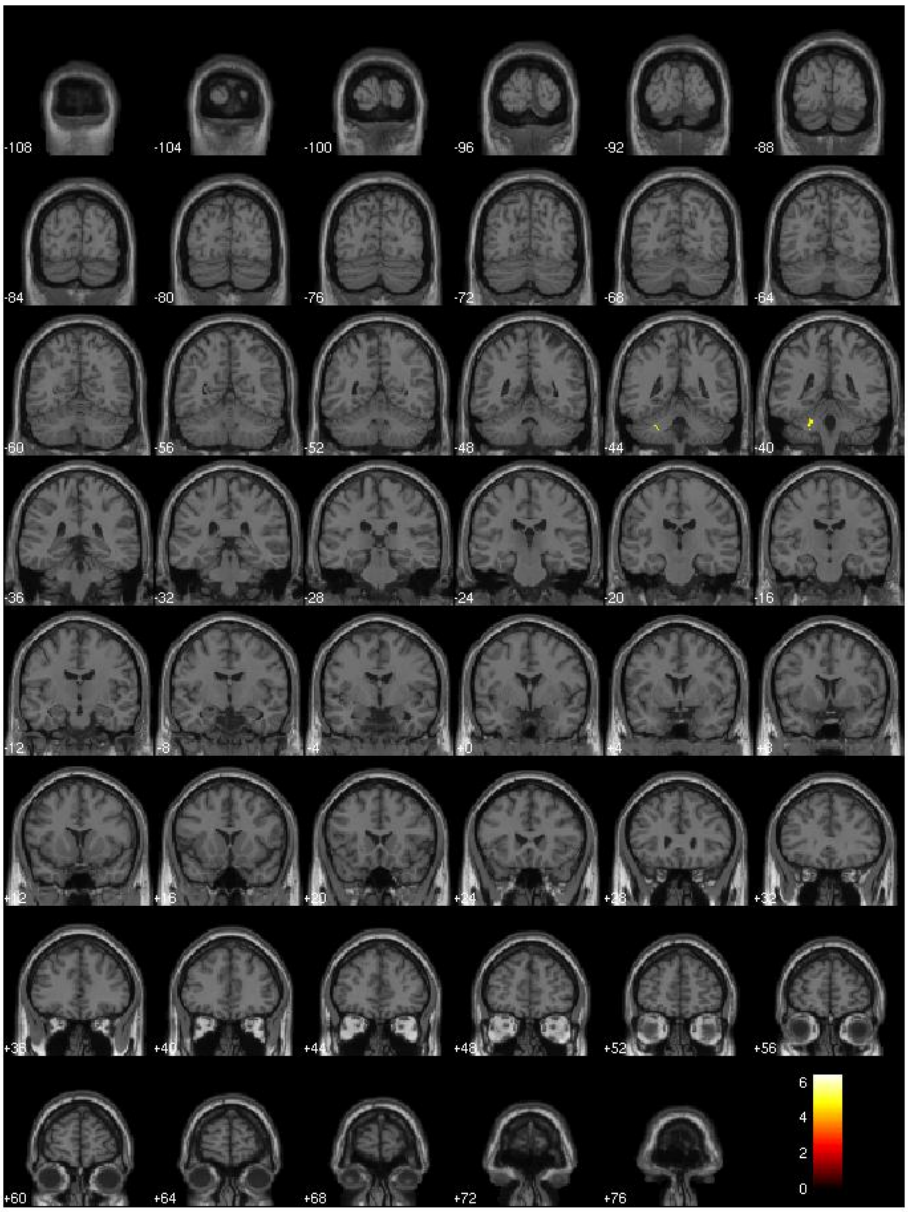
Cluster in the Autism group on the neutral versus baseline contrast with a significant positive correlation between brain activation and ADOS Calibrated Severity Score using a small volume correction centred on the area of significant activation identified in the faces > baseline contrast.

Cluster	$p_{FWE\text{-}corr}$	k_E	$(Z_{\equiv})$	x	y	z
Left cerebellum lobules IV / V	0.006	27	3.70	-20	-38	-30

Note. Only clusters of p<0.1 family-wise error-corrected significance are reported.

Figure A9

Cluster in the autism group on the neutral versus baseline contrast with a significant positive correlation between brain activation and ADOS Calibrated Severity Score using a small volume correction centred on the area of significant activation identified in the fear > baseline contrast.



Note. Only clusters of p<0.1 family-wise error-corrected significance are shown.

Appendix 7: Smith, McKechanie, Straathof *et al* (poster) (2019)

Similar impairments of Functional Connectivity in a Rat model and Humans with Fragile X Syndrome.

Poster presented by colleague Joanna Smith at The Simons Initiative for the Developing Brain 3rd research retreat, 12th-13th September 2019, Edinburgh, UK

Includes human data acquired as part of the FXS imaging study.
Analyses by Joanna Smith.

Similar impairments of Functional Connectivity in a Rat model and Humans with Fragile X Syndrome.

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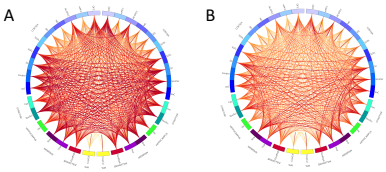
Abstract

A key requirement for the effective development of novel therapies for intellectual disability (ID) is the ability to directly compare findings from basic neuroscience in rodent models with human studies. Methods that can be applied to both human and rodents would facilitate this endeavour. Functional magnetic resonance imaging (fMRI) is a non-invasive system-wide imaging technique that is directly comparable across species. By measuring correlates of neuronal activity, fMRI provides a powerful tool to investigate how brain activity is modulated across different states, such as during development, disease or following a pharmacological intervention. Furthermore, resting state fMRI studies find similar functional connectivity networks in humans and rats, suggesting that this technique may provide a platform to compare humans and animal models of neurological conditions.

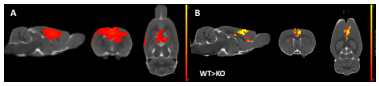
An advantage of using rat over mouse models for modelling neurodevelopmental disorders is that their brain size allows the use of fMRI. In this study we used a common imaging approach in individuals with FXS and *Fmr1* knockout rats that model the disorder, using a 3T and 7T scanner respectively. We assessed whether the loss of FMRP leads to similar differences in brain connectivity across species. Our findings from lightly anaesthetised *Fmr1*$^{-/y}$ and WT rats indicates a global decrease in functional connectivity. More specifically, we find a decrease in their Default Mode Network (DMN) connectivity. We observe a similar decrease in DMN connectivity in individuals with FXS. Furthermore, we find an increased connectivity selectively in the cingulate cortex of FX individuals compared to TD. This latter finding mirrors electrophysiological data from rodent model of Fragile X. In addition, preliminary findings suggest that early lovastatin treatment may have a positive effect on the development of Default Mode Network connectivity in adult *Fmr1* knockout rats. This study demonstrates the feasibility of using resting state fMRI to directly compare rat models and individuals with moderate to severe ID and ASD, and the potential for using fMRI as a biomarker for clinical trials.

1. *Fmr1* knockout rats are globally hypoconnected compared to their WT littermates



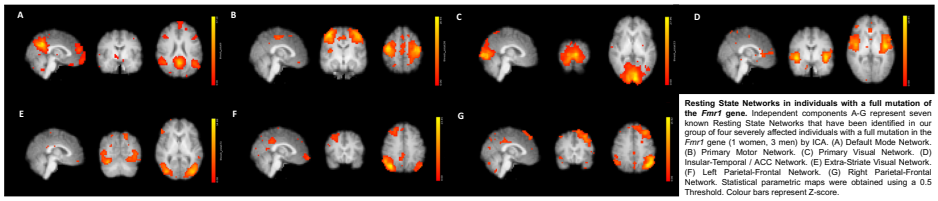
Functional connectogram in WT and *Fmr1*$^{-/y}$ LEH rats (3-4 months old) under light anaesthesia (1% isoflurane). (A-B) Functional connectogram between 34 brain regions across both hemispheres, in WT rats (A) (n=13) and *Fmr1*$^{-/y}$ rats (B) (n=11). Each line represents the functional connectivity between the two brain regions it links. Greater functional connectivity is represented by thicker and darker lines.

2. The Default Mode Network is hypoconnected in adult *Fmr1* knockout rats



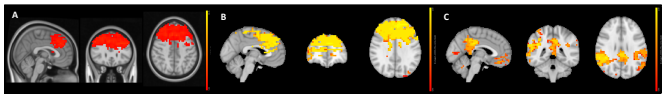
Between genotype comparison of the Default Mode Network identified in adult WT and *Fmr1*$^{-/y}$ LEH rats (3-4 months old) under light anaesthesia (1% isoflurane), identified by Independent Component Analysis. (A) represent the spatial maps of the Default Mode Network obtained by Independent Component Analysis (ICA) in our cohort (mixed genotype, n=24; 13 WT and 11 KO). Statistical parametric maps were obtained using a 0.5 Threshold. Colour bars represent Z-score. (B) Decreased connectivity in the DMN in *Fmr1*$^{-/y}$ compared to their WT littermates. Colour bar represent 1-P-value, corrected for multiple comparisons.

3. Resting State Networks are identifiable in individuals with severe Fragile X Syndrome



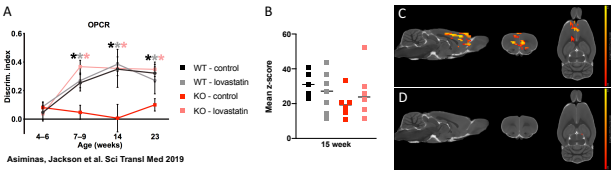
Resting State Networks in individuals with a full mutation of the *Fmr1* gene. Independent components A-G represent seven known Resting State Networks that have been identified in our group of four severely affected individuals with a full mutation in the *Fmr1* gene (1 women, 3 men) by ICA. (A) Default Mode Network. (B) Primary Motor Network. (C) Primary Visual Network. (D) Insular-Temporal / ACC Network. (E) Extra-Striate Visual Network. (F) Left Parietal-Frontal Network. (G) Right Parietal-Frontal Network. Statistical parametric maps were obtained using a 0.5 Threshold. Colour bars represent Z-score.

4. Local hyperconnectivity and long-range hypoconnectivity in the Default Mode Network of individuals with FXS



Increased local and decreased long-range connectivity in the Default Mode Network of individuals with Fragile X Syndrome. (A) Component encompassing the dorsal ACC and the superior frontal gyrus obtained by ICA (n= 5 FXS and 5 age-matched controls). (B) Greater connectivity in individuals with FXS compared to typically developing individuals. (C) Greater connectivity to the precuneus / posterior cingulate and lateral temporo-parietal lobe in typically developing individuals compared to FX individuals. Dual regression (B-C) was performed on 5 FXS and 17 controls. Colour bars represent TFCE-corrected 1-P-values, ranging from 0.95-1.

5. Early lovastatin treatment may have a positive effect on the development of Default Mode Network connectivity in adult *Fmr1* knockout rats



Asiminas, Jackson et al. Sci Transl Med 2019

Effect of early lovastatin treatment on the developmental trajectory of DMN functional connectivity in WT and *Fmr1*$^{-/y}$ rats. (A) Discrimination index at different ages for WT and *Fmr1*$^{-/y}$ rats treated with control or lovastatin diet in object-place-context recognition task. (B) DMN functional connectivity in *Fmr1*$^{-/y}$ rats and WT littermates at 15 week. From week 4-9, animals were either on a lovastatin or control diet. Squares represent animals' mean z-score, colour-coded by genotype and treatment received (n=6/group). (C) Decreased connectivity in the DMN of *Fmr1*$^{-/y}$ rats compared to their WT littermates at 8 week (ANOVA, genotype effect. TFCE-corrected 1-P-value ranging from 0.5-0.952). (D) No between-genotype difference in DMN connectivity at 15 week (TFCE-corrected 1-P-value <0.5).

Summary

- **The Default Mode Network is hypoconnected both in individuals with Fragile X Syndrome and in rat model of the disorder, supporting the translational value of resting-state fMRI.**
- *Fmr1* knockout rats were found to be globally hypoconnected compared to WT rats.
- Specifically, the Default Mode Network is hypoconnected in *Fmr1* knockout rats compared to their WT littermates.
- Known Resting State Networks can be identified in severely affected individuals with Fragile X Syndrome.
- The dorsal ACC and the superior frontal gyrus of the Default Mode Network of Fragile X individuals is locally hyperconnected, mirroring electrophysiological studies in rodent models of FXS.
- Preliminary findings suggest that early lovastatin treatment may have a positive effect on the development of Default Mode Network connectivity in adult *Fmr1* knockout rats .

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